SPONSOR EXECUTIVE SUMMARY

COLOGUARD™ sDNA-BASED COLORECTAL CANCER SCREENING TEST
EXACT SCIENCES CORPORATION

P130017

March 27, 2014

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

1.0 SUMMARY

1.1 Disease Background

Colorectal cancer (CRC) is a major public health problem and the second leading cause of cancer deaths in the United States.[1] More than 140,000 new cases are reported annually in the United States, and more than 50,000 people die from CRC-related causes each year.[2]

Colorectal adenocarcinoma, the most common form of CRC, develops over time from precancerous lesions. Typically, this begins with a small benign adenomatous polyp which develops over a 10 to 15 year period into a precancerous lesion, and subsequently into invasive CRC.[3]

Cases of CRC are typically categorized (staged) into one of four stages:

- Stage I – Localized; confined to primary site
- Stage II – Regional; ranges from invading muscle layer to spreading to nearby organs
- Stage III – Regional; spreads to regional lymph nodes
- Stage IV – Distant; cancer has metastasized.[4]

Because patients at earlier stages of CRC are often asymptomatic, CRC screening allows for early identification of cancer at stages where treatment is more likely to be effective. For this reason, identifying CRC at earlier stages can have a significant impact on mortality. Five-year survival rates for Stage I-II CRC cases are 94% and 82%, respectively.[5] Five-year survival declines to 67% for Stage III cases and is only 12% when CRC is identified in Stage IV. Screening also has the potential to decrease CRC incidence by as much as 90%,[6] since precancerous lesions identified during screening can be removed before developing into CRC.[7]

Current guidelines for CRC screening in the average-risk population recommend regular screening of both men and women starting at age 50.[8] Current screening for CRC includes both invasive and non-invasive tools. Invasive methods include conventional (optical) colonoscopy, flexible sigmoidoscopy, double contrast barium enema, and computed tomographic (CT) colonography. Current non-invasive methods include guaiac-based fecal occult blood testing (gFOBT) and immunochemical-based fecal occult blood testing (FIT). Screening guidelines recommend a number of different screening options. For example, the current US Preventive Services Task Force

---

[1] American Cancer Society: Cancer Facts and Figures, 2013.

[2] *Id.*

[3] Kelloff GJ, Schilsky RL, Alberts DS, et al. (2004) Colorectal adenomas: a prototype for the use of surrogate end points in the development of cancer prevention drugs. *Clin Cancer Res* 10(11):3908-3918.

[4] National Cancer Institute, http://www.cancer.gov/cancertopics/wyntk/colon-and-rectal/page8, accessed 1/7/2014.

[5] Lansdorp-Vogelaar I, van Ballegooijen M, Zauber AG, Habbema J, and Kuipers EJ. (2009) *J Natl Cancer Inst*; 101:1412-1422.

[6] *Winawer SJ, Zauber AG. (2002) The Advanced Adenoma as the Primary Target of Screening. Gastrointest Endosc Clin N Am. 12(1):1-9.*

[7] A.G. Zauber, I. Lansdorp-Vogelaar, A.B. Knudsen, J. Wilschut, M.v. Ballegooijen, and K.M. Kuntz. (2008) Evaluating Test Strategies for Colorectal Cancer Screening: A Decision Analysis for the U.S. Preventive Services Task Force. *Ann Intern Med* 149:659-69.

[8] Levin, Lieberman, McFarland, *et al*. (2008) Screening and surveillance for the early detection of colorectal cancer and adenomatous polyps, 2008: A Joint Guideline From the American Cancer Society, the US Multi-Society Task Force on Colorectal Cancer, and the American College of Radiology. *Gastroenterology* 134(5):1570-1595.

recommends patients undergo colonoscopy, high sensitivity fecal occult blood testing (FIT or FOBT) or sigmoidoscopy combined with high sensitivity FIT/FOBT.[9]

All patients who have a positive test, whether with a non-invasive test or invasive screening method (other than colonoscopy), warrant further investigation through a diagnostic colonoscopy to rule out the presence of and/or remove polyps or CRC.

Unfortunately, compliance with recommended CRC screening is suboptimal. Recent estimates suggest that approximately 1 in 3 adults are not undergoing screening as recommended.[10]

1.2 *Cologuard* sDNA-based CRC Screening Test

Cologuard is an *in vitro* diagnostic screening test that incorporates both stool DNA ("sDNA") and fecal immunochemical test ("FIT") techniques and is designed to analyze patients' stool samples for markers associated with the presence of CRC and precancerous lesions ("advanced adenomas" or "AA").[11]

Exact Sciences has been working to develop *Cologuard* for over 15 years. More recently, development was enabled by advances in science; recent research has identified more discriminant DNA markers that are associated with CRC and precancerous lesions and allow for detection of disease with higher analytical sensitivity.[12] Moreover, new selective enrichment and amplification techniques can detect even very small amounts of DNA markers in stool, and preservatives have been developed that can preserve DNA in stool for this later analysis. Finally, the ability to automate analysis of stool samples in the laboratory has reduced the potential for human error in sample analysis.

sDNA testing techniques examine a single stool sample for the presence of molecular markers of altered DNA that are contained in the cells shed by cancerous tumors and large polyps into the large bowel. The FIT technique detects the presence of hemoglobin in the same stool sample. Together these two techniques are combined in the *Cologuard* test algorithm to generate a single score that is compared to a pre-defined cutoff. Only a positive or negative result is reported; the individual marker results and quantitative score are not presented.

---

[9] U.S. Preventive Services Task Force. Screening for Colorectal Cancer: U.S. Preventive Services Task Force Recommendation Statement. *Ann Intern Med.* 2008;149:627-637; also available at: http://www.uspreventiveservicestaskforce.org/uspstf08/colocancer/colosum.htm.

[10] *Id.*

[11] *Cologuard*™ is a trademarked brand name that the company uses with the product.

[12] Melotte V, Lentjes MH, van der Bosch SM, et al. (2009) N-Myc downstream-regulated gene 4 (NDRG4): a candidate tumor suppressor gene and potential biomarker for colorectal cancer. *J Natl Cancer Inst* 101(13):916-27; Zou H, Harrington JJ, Shire AM, et al. (2007) Highly methylated genes in colorectal neoplasia: implications for screening. *Cancer Epidemiol Biomarkers Prev* 16(12):2686-96; Zou H, Allawi H, Cao X, et al. (2012) Quantification of methylated markers with a multiplex methylation-specific technology. *Clin Chem* 58(2):375-83; Ahlquist DA, Taylor WR, Yab TC, et al. (2012) Methylated Gene Markers in Stool: Effects of Demographic, Drug, Body Mass and other Patient Characteristics, in American Association for Cancer Research. Chicago, IL; Ahlquist DA, Zou H, Domanico M, et al. (2012) Next-generation stool DNA test accurately detects colorectal cancer and large adenomas. *Gastroenterol*; 142(2):248-56; Ahlquist DA, Taylor WR, Mahoney DW, et al. (2012) The stool DNA test is more accurate than the plasma septin 9 test in detecting colorectal neoplasia. *Clin Gastroenterol Hepatol*; 10(3):272-7; Lidgard GP, Domanico MJ, Bruinsma JJ, et al. (2013) Clinical performance of an automated stool DNA assay for detection of colorectal neoplasia. *Clin Gastroenterol Hepatol*; 11(10):1313-8; Heigh RI, Yab TC, Mahoney DW, et al. (2014) Screen Detection of Serrated Polyps by Stool DNA Multi-Target Testing: Comparisons against Fecal Immunochemical Occult Blood Testing. PLOS ONE 2014; DO1:10.1371/journal.pone.0085659.

Screening with *Cologuard* involves three steps: (1) Collection of stool by a patient with the *Cologuard* sample collection kit; (2) Laboratory analysis of the sample; (3) Application of an algorithm that produces a positive or negative result for the physician.

Cologuard is designed to detect three independent families of markers that exhibit an additive association with CRC and AA. The first DNA family targets epigenetic changes in the form of gene promoter region methylation. The second DNA family targets specific point mutations in *KRAS*. The third family of markers is non-DNA based and detects occult hemoglobin.

Unlike blood markers that are intermittently found in stool, the DNA markers are released from cells that regularly and continuously slough from the lining of the colon into the stool.[13] The specific DNA markers that *Cologuard* targets are *NDRG4* promoter region hypermethylation, *BMP3* promoter region hypermethylation, and seven *KRAS* gene point mutations. Additionally, Beta-actin ("*ACTB"*) is a reference gene used for confirmation and quantitative estimation of the total amount of human DNA present in each sample.

1.3 *Cologuard* Indications for Use

Cologuard is intended for use as an adjunctive screening test for the detection of colorectal neoplasia associated DNA markers and for the presence of occult hemoglobin in human stool. A positive result may indicate the presence of colorectal cancer or pre-malignant colorectal neoplasia. *Cologuard* is not intended as a replacement for diagnostic colonoscopy. *Cologuard* is intended to be used in conjunction with colonoscopy and other test methods in accordance with recognized screening guidelines. A positive result in *Cologuard*, as with any screening test, should be followed by colonoscopy. *Cologuard* is intended for patients who are typical candidates for colorectal cancer screening, adults of either sex, 50 years or older, who are at average risk for colorectal cancer.

1.4 Pivotal Study

The *Cologuard* pivotal study (Multi-Target Colorectal Cancer Screening Test for the Detection of Colorectal Advanced Adenomatous Polyps and Cancer study or "DeeP-C") was a prospective, multi-centered, trial in which the sensitivity (for CRC and AA) and specificity of *Cologuard* in the average-risk screening population were determined. Results were compared to the results of an optical colonoscopic examination and all biopsied and/or excised lesions were subjected to histopathology. Additionally, the study evaluated the performance of *Cologuard* compared to FIT for CRC and AA detection. The study was designed based on input of medical experts, the FDA and the Centers for Medicare & Medicaid Services

The study enrolled a total of 12,766 subjects at 90 sites, including both primary care point-of-referral sites and colonoscopy centers. Subjects eligible for enrollment in the study were those between the ages of 50 and 84 years (inclusive), who were at average risk for development of CRC and asymptomatic for gastrointestinal symptoms warranting diagnostic colonoscopy.

Enrolled subjects were categorized as having CRC, AA, non-advanced adenomas or as negative, based on their most clinically significant lesion confirmed by histopathologic analysis. Further information regarding the categories used is provided in Section 6.0 below.

---

[13] Ahlquist DA, McGill DB, Fleming JL, et al. (1989) Patterns of occult bleeding in asymptomatic colorectal cancer. *Cancer*; 63(9):1826-30; Ahlquist DA, Harrington JJ, Burgart LJ, Roche PC. (2000) Morphometric analysis of the "mucocellular layer" overlying colorectal cancer and normal mucosa: relevance to exfoliation and stool screening. *Hum Pathol*; 31(1):51-7.

The DeeP-C population closely mirrored the general CRC screening population in the United States, as described in the literature.[14] DeeP-C had a larger percentage of Hispanic/Latino subjects, compared with the U.S. CRC screening population (9.9% and 2.1%, respectively), but was otherwise generally representative of the CRC screening population.

Given that the diagnostic tests applied to all study subjects were non-invasive and were followed by standard colonoscopy, adverse events ("AEs") were rare. Therefore, no formal statistical analyses for safety were planned, but events reported during the study are described below.

The primary endpoint of the study was the sensitivity (for CRC) and specificity of *Cologuard*, using colonoscopy with histopathology as the reference method. The primary analysis required the one-sided 95% lower bound of the sensitivity of *Cologuard* for CRC to exceed 65%. The co-primary analysis of specificity required that the lower specificity bound for Cologuard exceed 85%. The secondary endpoints of the study compared *Cologuard* to FIT for detection of CRC and AA, seeking to establish non-inferiority for CRC sensitivity and superiority for AA sensitivity. Testing for superiority with respect to CRC sensitivity was permitted if non-inferiority was established.

1.4.1 Effectiveness Results

Results from the DeeP-C study demonstrated that *Cologuard* successfully met the primary endpoint of the study, substantially exceeding the pre-specified criteria for study sensitivity and meeting expectations for specificity. Moreover, the study results demonstrated superiority of *Cologuard* compared to FIT for both CRC and AA detection.

As shown in the table below, *Cologuard* sensitivity for CRC was 92.3% (60/65) with a one-sided 95% confidence interval lower bound of 84.5%, well above the pre-specified threshold for study success (65%). Thus, the study was a success with respect to sensitivity for CRC.

	Valid *Cologuard* (N=65) Positive Result
Case Category, n/N (%)	
1: CRC Stages 1-4	92.3% (60/65)
Sensitivity Based on Category 1: Primary (one-sided 95% lower bound)	92.3% (>84.5%)
Sensitivity Based on Category 1: Supportive (two-sided 95% % lower bound)	92.3% (>83.0%)

[1] Percentages based on valid test results within a category.
[2] Lower bounds calculated using an exact one-sided binomial test.

Similarly, Cologuard specificity was 86.6% with a one-sided 95% confidence interval lower bound of 86.0%, above the pre-specified threshold for study success (85%). Thus, the study was a success with respect to specificity.

[14] Schenck AP, Peacock SC, Klabunde CN, Lapin P, Coan JF, Brown ML. (2009) Trends in Colorectal Cancer Test Use in the Medicare Population, 1998–2005. *Am J Prev Med* 37(1).

	Valid *Cologuard* (N=9198) Negative Result
Case Category, n/N (%)	
3: 1-2 Adenomas 5-<10 mm	607/749 (81.0%)
4: ≥3 Adenomas <10 mm, Non-advanced	302/419 (72.1%)
5: 1-2 Adenomas ≤5 mm, Non-advanced	1496/1735 (86.2%)
6.1: Negative upon histopathological review	1543/1821 (84.7%)
6.2: No findings on colonoscopy, no histopathological review	4019/4474 (89.8%)
Specificity Based on Categories 3-6: Primary (one-sided 95% lower bound)	86.6% (>86.0%)
Specificity Based on Categories 3-6: Supportive (one-sided 97. 5% lower bound)	86.6% (>85.9%)

[1] Percentages based on valid test results within a category.
[2] Lower bounds calculates using an exact one-sided binomial test.

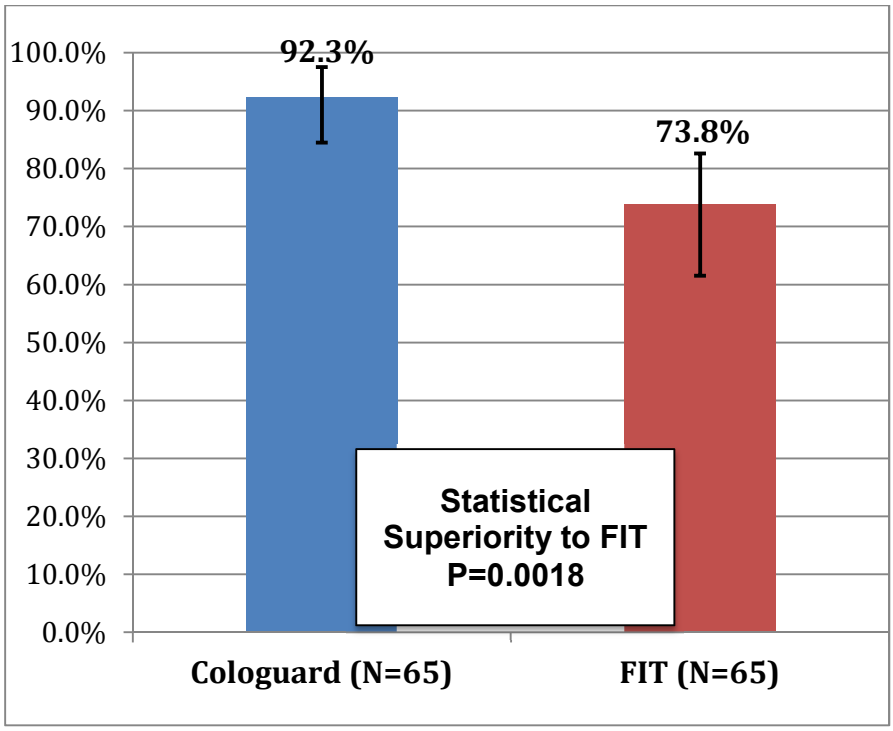
Therefore, Cologuard met both primary endpoint analysis thresholds and, as a result, the DeeP-C study can be declared a success.

In addition, *Cologuard* was compared to FIT for CRC and AA sensitivity. Specifically, the comparison to FIT was evaluated to establish both non-inferiority and superiority with respect to CRC detection, as well as superiority with respect to AA detection.

The secondary endpoint analysis demonstrates that *Cologuard* is statistically superior to FIT with respect to detection of both CRC and AA. As shown in **Figure 1** below, sensitivity of *Cologuard* for CRC was 92.3% (60/65), compared with 73.8% (48/65) for FIT (two-sided McNemar p-value=0.0018). Further, as shown in **Figure 2** below, sensitivity of *Cologuard* for AA was 42.4% (321/757) compared with 23.8% (180/757) for FIT (p<0.0001).

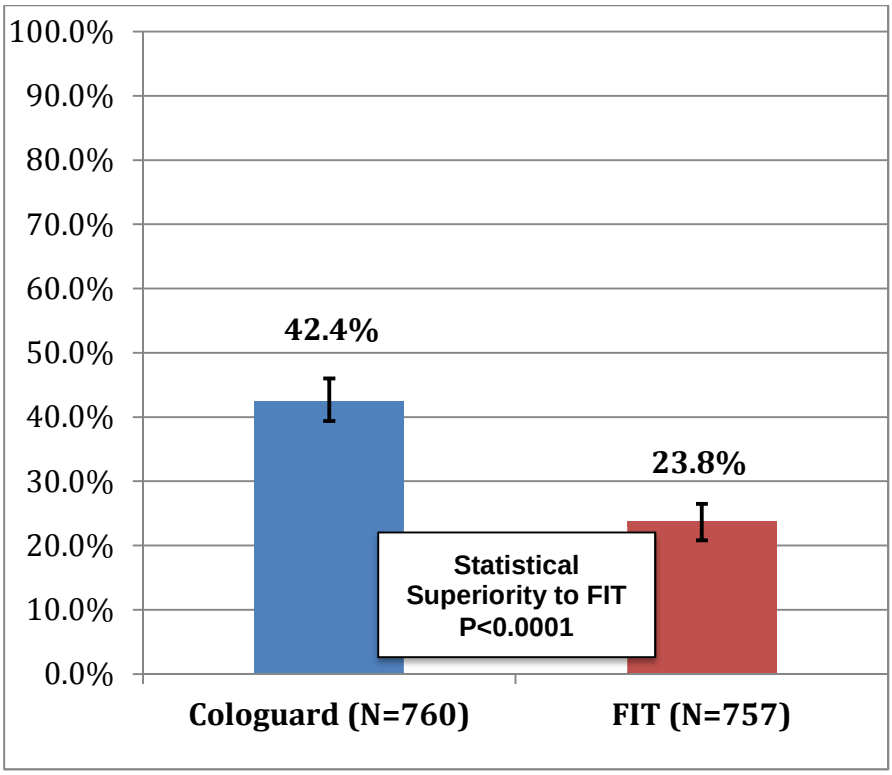
Notably, *Cologuard* correctly captured 60 of the 65 total CRC cases identified by colonoscopy (92.3%), while FIT captured only 48 of the 65 CRC cases identified by colonoscopy (73.8%). FIT identified only a single cancer that was not identified by *Cologuard*, whereas *Cologuard* identified 13 cancers that were missed by FIT.

Figure 1: Secondary Endpoint - CRC Comparison to FIT



As noted above and shown **Figure 2**, *Cologuard* was also found superior to FIT for AA detection. *Cologuard* sensitivity for AA was 42.4% compared with 23.8% for FIT. Notably, FIT identified only 29 AA cases that were not captured by *Cologuard*, while *Cologuard* identified 170 AA cases that were not positive on the FIT test. These results successfully demonstrated superiority for *Cologuard* over FIT for AA detection (one-sided McNemar p-value < 0.0001 for superiority).

Figure 2: Secondary Endpoint - AA Comparison to FIT



Cologuard sensitivity for AA represented a clear advantage over FIT, particularly for larger adenomas. Additionally, Cologuard sensitivity for adenoma with carcinoma in situ/high grade dysplasia, lesions at high risk of developing into CRC, was 69.2% compared with 46.2% for FIT.[15] *Cologuard* also provides for detection of serrated lesions, a lesion that has been increasingly linked to CRC development and that has been difficult for FIT to detect due to the fact that the lesions do not bleed. Cologuard sensitivity for serrated lesions was 43.0%, compared with 5.1% for FIT.

A comparison to FIT with respect to specificity was not performed, as the two tests are designed to have different specificities. However, the company compared the number of true negatives captured by *Cologuard* out of those identified by colonoscopy (7,936/9,167, 86.6%), to those captured by FIT. FIT correctly identified more true negatives, (8,695/9,167 94.9%). Cologuard was designed to maximize sensitivity for CRC and AA, while maintaining a clinically acceptable specificity, as described in more detail in Section 6.0.

1.4.1 Safety

With respect to safety, there were 4 adverse events ("AEs") reported and all were categorized as mild events, per the protocol definitions of AE severity. One subject broke a fingernail trying to open the collection kit, one subject cut his/her right thumb by using a knife to open the preservative bottle, one subject fell during stool collection, and one subject experienced a sprained hand during sample collection. There were no serious adverse events ("SAEs").

---

[15] Toll AD, Fabius D, Hyslop T, Pequignot E, DiMarino AJ, Infantolino A, Palazzo JP. (2011) Prognostic significance of high-grade dysplasia in colorectal adenomas. *Colorectal Dis*;13(4):370-3.

One subject died after providing a stool sample but prior to undergoing colonoscopy, due to narcotic and ethanol intoxication. This event was deemed unrelated to the study and not captured as an AE because it occurred outside of the protocol defined AE reporting period.

1.5 Conclusion

The results of the DeeP-C study clearly demonstrated that *Cologuard* met the primary endpoints of the study, establishing a clinically meaningful sensitivity and specificity that exceeded the protocol-specified threshold for study success.

Cologuard also met the secondary endpoints of the study, powerfully demonstrating both non-inferiority and superiority compared to FIT for CRC sensitivity and superiority for AA sensitivity.

2.0 LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

CRC – Colorectal cancer

AA – Advanced Adenoma

FIT – Fecal Immunochemical Test (i.e. OC FIT-CHEK, Polymedco, Inc.)

AE – Adverse event. Adverse events were defined, in accordance with international guidelines, as a new, undesirable medical experience or worsening of a pre-existing condition, which occurred during study participation, regardless of its relationship to the study device, the procedure, or the medications used.

CI – Confidence interval

sDNA – stool DNA

SAP – Statistical analysis plan

CRF – Case Report Form

PPV – Positive Predictive Value

NPV – Negative Predictive Value

PLR – Postive Likelihood Ratio

NLR - Negative Likelihood Ratio

SAE – Serious adverse event. An adverse event of any severity (mild, moderate or severe) which leads to hospitalization, lengthening of hospitalization, permanent disability, congenital defect or death.

DeeP-C – Multi-Target Colorectal Cancer Screening Test for the Detection of Colorectal Advanced Adenomatous Polyps and Cancer study

FOBT – Fecal occult blood test

3.0 CRC BACKGROUND AND CURRENT SCREENING MODALITIES

Colorectal cancer (CRC) is a major public health problem and the second leading cause of cancer deaths in the United States.[16] More than 140,000 new cases are reported annually in the United States, and more than 50,000 people die from CRC-related causes each year.[17] A 2012 report from the American Cancer Society (ACS) estimated that 1 in 19 males and 1 in 20 females will develop CRC during his or her lifetime.

Colorectal adenocarcinoma, the most common form of CRC, arises from the epithelial cells that line the lumen of the colon and rectum. Colorectal adenocarcinoma results from a multistep process of colorectal epithelial carcinogenesis that evolves over a number of years. Colorectal adenocarcinoma typically begins as a small benign adenomatous polyp which, over 10 to 15 years period grows, develops advanced adenoma features (indicators of increased likelihood of evolution to CRC) and evolves into invasive CRC. This evolution from a benign polyp into a precancerous lesion or advanced adenoma ("AA") has led some researchers to assert that AAs should be a primary target of CRC screening, since precancerous lesions identified during screening can be removed before developing into CRC.[18]

CRC at its earliest stages is confined within the wall of the colon (tumor-node-metastasis Stages I and II) and may spread, if untreated, to regional lymph nodes (Stage III) and further metastasize to distant sites (Stage IV).

Because patients with CRC are often asymptomatic at early stages, CRC screening allows for early identification of cancer at stages where treatment is more likiely to be effective. For this reason, identifying CRC at earlier stages can have a significant impact on mortality. Five-year survival rates for Stage I-II CRC cases are 94% and 82%, respectively.[19] Five-year survival declines to 67% for Stage III cases and is only 12% when CRC is identified in Stage IV. Screening also decreases CRC incidence by as much as 90%,[20] due to the removal of precancerous lesions. Removing an adenoma has been estimated to reduce the risk of developing CRC up to 10 years later to equal that of a person without any adenoma findings.[21]

Current guidelines for CRC screening in the average-risk population recommend regular screening of both men and women starting at age 50, as the incidences of both CRC and premalignant lesions increase sharply after this age.

Conventional screening for CRC includes both invasive and non-invasive tools. Invasive tools include flexible sigmoidoscopy, double contrast barium enema, computed tomographic (CT) colonography and optical colonoscopy. Current non-invasive CRC screening tools include guaiac-

---

[16] American Cancer Society: Cancer Facts and Figures, 2013.

[17] *Id.*

[18] Winawer SJ, Zauber AG. (2002) The Advanced Adenoma as the Primary Target of Screening. *Gastrointest Endosc Clin N Am.* 12(1):1-9; A.G. Zauber, I. Lansdorp-Vogelaar, A.B. Knudsen, J. Wilschut, M.v. Ballegooijen, and K.M. Kuntz. (2008) Evaluating Test Strategies for Colorectal Cancer Screening: A Decision Analysis for the U.S. Preventive Services Task Force. *Ann Intern Med* 149:659-69.

[19] Lansdorp-Vogelaar I, van Ballegooijen M, Zauber AG, Habbema J, and Kuipers EJ. (2009) *J Natl Cancer Inst*; 101:1412-1422.

[20] *Winawer SJ, Zauber AG. (2002) The Advanced Adenoma as the Primary Target of Screening. Gastrointest Endosc Clin N Am. 12(1):1-9.*

[21] Zauber AG, Winawer SJ, O'Brien MJ (2012). Colonoscopic Polypectomy and Long-Term Prevention of Colorectal-Cancer Deaths. *NEJM* 366:687-96.

based fecal occult blood testing (gFOBT) and immunochemical-based fecal occult blood testing (FIT).

The current US Preventive Services Task Force recommends patients undergo one of three CRC screening regimens:

1. Screening colonoscopy every 10 years
2. High-sensitivity fecal occult blood test (FOBT or FIT) annually
3. Sigmoidoscopy every 5 years combined with high sensitivity FOBT/FIT every 3 years.[22]

Other guidelines provide similar recommendations, including some which more specifically recommend use of FIT.[23] Comparisons of FIT and gFOBT indicate that FIT sensitivity for CRC is higher than that of traditional gFOBT tests with comparable specificity.[24]

All patients who have a positive test with any non-invasive screening method or invasive (other than colonoscopy) test warrant further investigation through a diagnostic colonoscopy to rule out the presence of and/or remove polyps or CRC.

Colonoscopy, as it is the final diagnostic pathway for all other screening tests, is considered to be the "reference standard" for CRC screening and its use has expanded rapidly in the past decade.[25] In contrast to sigmoidoscopy, which only examines the distal colon and rectum, the entire colorectal lining is directly examined visually using a colonoscope. Precancerous and cancerous growths throughout the colon can be found and either removed or biopsied.

With respect to the invasive screening methods other than colonoscopy, flexible sigmoidoscopy involves use of a flexible sigmoidoscope, through which a clinician can examine the rectum and distal colon for precancerous and cancerous growths. Flexible sigmoidoscopy can discover up to 65% of polyps, with a low rate of complications.[26,27] Most of the available studies have suggested that regular screening with sigmoidoscopy after the age of 50 can help identify an increased number of early cases and a corresponding reduction in the number of deaths from CRC when compared to a nonscreening environment.[28] In the NIH-sponsored Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial, use of flexible sigmoidoscopy was found to reduce overall CRC

---

[22] U.S. Preventive Services Task Force. Screening for Colorectal Cancer: U.S. Preventive Services Task Force Recommendation Statement. *Ann Intern Med.* 2008;149:627-637; also available at: http://www.uspreventiveservicestaskforce.org/uspstf08/colocancer/colosum.htm.

[23] Levin B, Lieberman DA, McFarland B, et al. (2008) Screening and surveillance for the early detection of colorectal cancer and adenomatous polyps, 2008: A joint guideline from the American Cancer Society, the US Multi-Society Task Force on Colorectal Cancer, and the American College of Radiology. CA Cancer J Clin 60:190-207.

[24] Whitlock EP, JS Lin, E Liles, et al. (2008) Screening for colorectal cancer: a targeted, updated systematic review for the US Preventive Services Task Force. *Ann Intern Med* 149: 638-658.

[25] American Cancer Society, Colorectal Cancer Facts & Figures, 2011-2013, available at http://www.cancer.org/acs/groups/content/@epidemiologysurveilance/documents/document/acspc-28323.pdf.

[26] National Cancer Institute: PDQ® Colorectal Cancer Screening. Bethesda, MD: National Cancer Institute. Available at:http://www.cancer.gov/cancertopics/pdq/screening/colorectal/HealthProfessional/page1/AllPages/Print#Section_32. Accessed 04/17/2012.

[27] Serious complications evaluated included perforations, hemorrhage, diverticulitis, cardiovascular events, severe abdominal pain, and death.

[28] PDQ® Cancer Information Summary. National Cancer Institute; Bethesda, Maryland. Colorectal Cancer Screening—Health Professional. Date last modified: 09/30/2011. Accessed 12/19/2011.

mortality by 26% and reduce the incidence of CRC by 21%.[29] Although sensitivity was not reported in the PLCO study, the estimated sensitivity of flexible sigmoidoscopy has been reported elsewhere as 58% to 75% for CRC;[30] however, sigmoidoscopy does not reach the right colon and has no impact on CRC mortality from proximal cancers.[31] Differences in the examiner's skill may be a factor contributing to the range of these estimates.[32] Specificity for CRC detection in the PLCO trial was 80% for men and 87% for women.[33] In their recent review of colon cancer screening methods, Whitlock et al. estimated a rate of serious complications, such as infection or bowel tear, of only 0.34 per 1,000 procedures.[34]

Double-contrast barium enema (DCBE) is very rarely used today for CRC screening and is no longer included in the US Preventive Services Task Force (USPSTF) guidelines (2008).[35] This screening method to detect polyps and colon cancers has a low complication rate, with a rate of perforation of about one in 25,000 examinations.[36] However, the sensitivity of a double contrast barium enema ranges from 39% to 90%, depending on the skill of the radiologist and on the preparation of the patient.[37] When compared to colonoscopy, screening with barium enema demonstrated a ten-fold increased miss rate, supporting colonoscopic examination as a more sensitive method.[38]

---

[29] Schoen, RE, Pinsky PF, Weissfeld JL, et al. (2012) Colorectal-Cancer Incidence and Mortality with Screening Flexible Sigmoidoscopy. *NEJM* 366(25): 2345-57.

[30] Whitlock EP, JS Lin, E Liles, et al. (2008) Screening for colorectal cancer: a targeted, updated systematic review for the US Preventive Services Task Force. *Ann Intern Med* 149: 638-658.

[31] Schoen, RE, Pinsky PF, Weissfeld JL, et al. (2012) Colorectal-Cancer Incidence and Mortality with Screening Flexible Sigmoidoscopy. *NEJM* 366(25): 2345-57.

[32] Whitlock EP, JS Lin, E Liles, et al. (2008) Screening for colorectal cancer: a targeted, updated systematic review for the US Preventive Services Task Force. *Ann Intern Med* 149: 638-658.

[33] Schoen, RE, Pinsky PF, Weissfeld JL, et al. (2012) Colorectal-Cancer Incidence and Mortality with Screening Flexible Sigmoidoscopy. *NEJM* 366(25): 2345-57.

[34] Serious complications evaluated included perforations, hemorrhage, diverticulitis, cardiovascular events, severe abdominal pain, and death. *See* Whitlock EP, JS Lin, E Liles, et al. (2008) Screening for colorectal cancer: a targeted, updated systematic review for the US Preventive Services Task Force. *Ann Intern Med* 149: 638-658.

[35] U.S. Preventive Services Task Force. Screening for Colorectal Cancer: U.S. Preventive Services Task Force Recommendation Statement. *Ann Intern Med.* 2008;149:627-637.

[36] Johns Hopkins Medicine, Colorectal Cancer (website). Available at http://www.hopkinscoloncancercenter.org/CMS/CMS_Page.aspx?CurrentUDV=59&CMS_Page_ID=2AB211EA-18D0-40BB-A8E5-B4096012E444.

[37] *See* Whitlock EP, JS Lin, E Liles, et al. (2008) Screening for colorectal cancer: a targeted, updated systematic review for the US Preventive Services Task Force. *Ann Intern Med* 149: 638-658; PDQ® Cancer Information Summary. National Cancer Institute; Bethesda, Maryland. Colorectal Cancer Screening—Health Professional. Date last modified: 09/30/2011. Accessed 12/19/2011. *See also* Ramos C, De Jesús-Caraballo J, Toro DH, et al. (2009) Is barium enema an adequate diagnostic test for the evaluation of patients with positive fecal occult blood? *Bol Asoc Med P R.* 101(2):23-8 (reporting a sensitivity of 45% and a specificity of 90% for all adenomas); Johnson CD, MacCarty RL, Welch TJ, et al. (2004) Comparison of the relative sensitivity of CT colonography and double-contrast barium enema for screen detection of colorectal polyps. *Clinical Gastroenterol Hepatol*. 2(4):314-21 (reporting a sensitivity for double contrast barium enema of between 39% and 56% for polyps ≥1cm).

[38] Maheswaran T, Tighe R. (2011) Colorectal/anorectal: Colorectal cancer rates following a barium enema or colonoscopy. *Gut* 60(Suppl 1):A69-A70; Johns Hopkins Medicine, Colorectal Cancer (website). Available at http://www.hopkinscoloncancercenter.org/CMS/CMS_Page.aspx?CurrentUDV=59&CMS_Page_ID=2AB211EA-18D0-40BB-A8E5-B4096012E444. *See also* Ramos C, De Jesús-Caraballo J, Toro DH, et al. (2009) Is barium enema an adequate diagnostic test for the evaluation of patients with positive fecal occult blood? *Bol Asoc Med P R.* 101(2):23-8; Johnson CD, MacCarty RL, Welch TJ, et al. (2004) Comparison of the relative sensitivity of CT colonography and double-contrast barium enema for screen detection of colorectal polyps. *Clinical Gastroenterol Hepatol*. 2(4):314-21.

Computed Tomographic (CT) colonography (CTC), also known as "virtual" colonoscopy, is a radiologic approach to CRC screening. CTC can detect polyps and CRC throughout the entire colon and rectum. Patients found to have colorectal lesions on CTC are referred for a diagnostic optical colonoscopy for evaluation. The range of reported sensitivity and specificity rates of CTC varies widely, though many published studies found CT colonography to be highly specific in the detection of polyps. In a recent meeting of FDA's Gastroenterology and Urology Panel and Radiological Devices Panel, panelists generally concurred that CTC is just as effective for screening of large polyps as conventional colonoscopy.[39] Additionally, a review of the two largest studies of CTC reported pooled sensitivity for large adenomas (≥10 mm) of 92%, compared with an estimated 87.5% for colonoscopy.[40] Other studies have also reported sensitivity rates in the 90% range for large adenomas and cancer.[41] However, some studies have reported sensitivity rates ranging from 55% to 88% for large adenomas (≥10 mm).[42] Sensitivity estimates for smaller adenomas (≥6 mm) have been varied (ranging from 39% to 88.7%) and less comparable to colonoscopy (92.3%); specificity estimates range from 79.6% to 88% for these lesions.[43] This variability could be due to a number of factors.[44] Few serious procedure-related harms have been reported in CT colonography screening studies, including very low rates of perforation.[45]

With respect to non-invasive screening tools, Guaiac-based fecal occult blood testing (gFOBT) detects occult blood in a stool sample through a colorimetric reaction that depends on the iron moiety in the heme portion of the hemoglobin molecule. Occult blood may be related to bleeding from ulcerated CRCs and, less frequently, from polyps. Bleeding from CRCs and polyps, if present, may be intermittent, requiring frequent testing.[46] Generally, gFOBT must be performed on three (3) consecutive stool samples. Further, gFOBT is not specific for human blood and requires dietary restrictions and medication changes prior to the test to avoid false positive results. Finally, gFOBT has a low sensitivity for CRC, yielding only a modest reduction in mortality due to CRC.[47,48] Newer formulations of gFOBT ("high sensitivity gFOBT"), have been developed to address the technical sensitivity issues and may be similar to fecal immunochemical blood tests (FIT) in performance (see below). These higher sensitivity gFOBTs have now replaced the older formulations in the USPSTF

---

[39] Darcey, FDA Gastroenterology, Radiology Advisors Finally Agree: CT Colonography Benefits Outweigh Risks, The Gray Sheet, September 2013.

[40] Pickhardt PJ, Choi JR, Hwang I, et al. (2003) Computed tomographic virtual colonoscopy to screen for colorectal neoplasia in asymptomatic adults. *NEJM* 349:2191-200; Johnson CD, Chen MH, Toledano AY, et al. (2008) Accuracy of CT colonography for detection of large adenomas and cancers. *NEJM* 359:1207-17.

[41] Johnson CD, Chen MH, Toledano AY, et al. (2008) Accuracy of CT colonography for detection of large adenomas and cancers. *NEJM* 359:1207-17.

[42] de Haan MC, Halligan S, Stoker J. (2012) Does CT colonography have a role for population-based colorectal cancer screening? *Eur Radiol* 22(7):1495-503; Cotton PB, Durkalski VL, Pineau BC, et al. (2004) Computed tomographic colonography (virtual colonoscopy): a multicenter comparison with standard colonoscopy for detection of colorectal neoplasia. *JAMA* 291(14):1713-9.

[43] Whitlock EP, JS Lin, E Liles, et al. (2008) Screening for colorectal cancer: a targeted, updated systematic review for the US Preventive Services Task Force. *Ann Intern Med* 149: 638-658.

[44] Mulhall BP, Veerappan GR, Jackson JL. (2005) Meta-analysis: computed tomographic colonography. *Ann Intern Med.* 142(8):635-50.

[45] Whitlock EP, JS Lin, E Liles, et al. (2008) Screening for colorectal cancer: a targeted, updated systematic review for the US Preventive Services Task Force. *Ann Intern Med* 149: 638-658.

[46] Ahlquist DA, McGill DB, Fleming JL, et al. (1989) Patterns of occult bleeding in asymptomatic colorectal cancer. *Cancer*; 63(9):1826-30.

[47] Ransohoff, DF. (2007) What is the role of iFOBT in screening for colorectal cancer? *Gut* 56(10):1343–1344.

[48] Mandel JS, Bond JH, Church TR, et al. (1993) Reducing mortality from colorectal cancer by screening for fecal occult blood. *NEJM* 328(19):1365-71; Mandel JS, Church TR, Bond JH, et al. (2000) The effect of fecal occult-blood screening on the incidence of colorectal cancer. *NEJM* 343(22):1603-1607.

guidelines.[49] All fecal occult blood tests are non-specific for CRC or polyps in that they detect bleeding from any source such as hemorrhoids, inflammations and infection. These tests are typically used annually and data reported in the literature relates to annual screening. Screening performed less than annually may result in diminished effectiveness.

The fecal immunochemical test (FIT) is another non-invasive screening tool, which uses an antibody targeted to the globin portion of the hemoglobin molecule to identify occult blood. FIT offers several advantages over gFOBT, including no need for a change in diet or medication and single sample testing. FIT is less affected by upper gastrointestinal bleeding.[50] As each manufacturer's anti-hemoglobin antibody identifies a different epitope on the hemoglobin molecule, there are inherent differences in the performance characteristics of various tests. In recent comparisons, the sensitivity for CRC of FIT has been higher than that of traditional gFOBT tests with comparable specificity.[51] In general, FIT sensitivity for CRC ranges from 60-70% and for advanced adenomas from 20-30% when evaluated in the screening setting using colonoscopy as the reference standard.[52] Of note, FIT appears to detect distal neoplasms better than proximal ones.[53] FIT has been endorsed for CRC screening in recent multi-society guidelines.[54] The effectiveness of FIT, like gFOBT, depends upon repeated annual screening over time.

Unfortunately, despite the screening guideline recommendations, compliance with CRC screening is suboptimal. Recent estimates suggest that approximately 1 in 3 adults are not undergoing screening as recommended.[55] In addition, recent research indicates that adherence to screening guidelines increases when patients are given a choice of screening option.[56] Given that identification of precancer is important for reduction of CRC incidence and that detection of CRC at early stages has the potential to significantly reduce mortality, an optimal new screening tool would have high sensitivity for CRC and also effectively identify precancer. For maximum effectivness, a new screening tool also should have a potential for high patient compliance. *Cologuard* was designed to address these optimal test characteristics, as described further in the section that follows.

---

[49] U.S. Preventive Services Task Force. Screening for Colorectal Cancer: U.S. Preventive Services Task Force Recommendation Statement. *Ann Intern Med.* 2008;149:627-637.

[50] Morikawa T, Kato J, Yamaji Y. et al. (2005) A comparison of the immunochemical fecal occult blood test and total colonoscopy in the asymptomatic population. *Gastroenterology* 129(2):428-8.

[51] Whitlock EP, JS Lin, E Liles, et al. (2008) Screening for colorectal cancer: a targeted, updated systematic review for the US Preventive Services Task Force. *Ann Intern Med* 149: 638-658.

[52] Levin B, Lieberman DA, McFarland B, et al. (2008) Screening and surveillance for the early detection of colorectal cancer and adenomatous polyps, 2008: A joint guideline from the American Cancer Society, the US Multi-Society Task Force on Colorectal Cancer, and the American College of Radiology. *CA Cancer J Clin* 60:190-207 (citing Morikawa T, Kato J, Yamaji Y. et al. (2005) A comparison of the immunochemical fecal occult blood test and total colonoscopy in the asymptomatic population. *Gastroenterology* 129(2):428-8).

[53] Morikawa T, Kato J, Yamaji Y. et al. (2005) A comparison of the immunochemical fecal occult blood test and total colonoscopy in the asymptomatic population. *Gastroenterology* 129(2):428-8.

[54] Levin B, Lieberman DA, McFarland B, et al. (2008) Screening and surveillance for the early detection of colorectal cancer and adenomatous polyps, 2008: A joint guideline from the American Cancer Society, the US Multi-Society Task Force on Colorectal Cancer, and the American College of Radiology. *CA Cancer J Clin* 60:190-207.

[55] *Id.*

[56] Inadomi JM, Vijan S, Janz NK, Fagerlin A, Thomas JP, Lin YV, Muñoz R, Lau C, Somsouk M, El-Nachef N, Hayward RA. (2012) Adherence to colorectal cancer screening: a randomized clinical trial of competing strategies. *Arch Intern Med*; 172(7):575-82.

4.0 DEVICE DESCRIPTION

4.1 Intended Use/Indications for Use

Cologuard is intended for use as an adjunctive screening test for the detection of colorectal neoplasia associated DNA markers and for the presence of occult hemoglobin in human stool. A positive result may indicate the presence of colorectal cancer or pre-malignant colorectal neoplasia. *Cologuard* is not intended as a replacement for diagnostic colonoscopy. *Cologuard* is intended to be used in conjunction with colonoscopy and other test methods in accordance with recognized screening guidelines. A positive result in *Cologuard*, as with any screening test, should be followed by colonoscopy. *Cologuard* is intended for patients who are typical candidates for colorectal cancer screening, adults of either sex, 50 years or older, who are at average risk for colorectal cancer.

4.2 Test Design

Cologuard was designed to provide a test with good sensitivity for both CRC and AA. The test incorporates both sDNA and FIT techniques to analyze patients' stool samples for markers associated with the presence of CRC and AA. The test generates a single score based on the detection of hemoglobin and multiple DNA methylation and mutational markers, together with an assessment of the total amount of human DNA in each sample. This panel of complementary informative markers increases the likelihood of detection of cancerous or precancerous lesions, given the molecular heterogeneity of colorectal neoplasia. *Cologuard* consists of optimized sample processing and proprietary assay methodologies for detection of the following informative markers in stool:

- Fecal hemoglobin;
- *NDRG4* (N-myc downregulated gene 4) promoter DNA region hyper-methylation;
- *BMP3* (bone morphogenetic protein 3) promoter DNA region hyper-methylation;
- *KRAS* (V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog) gene DNA point mutations (7) (referred to as *KRAS1 and KRAS2*); and
- Total human DNA as measured using *ACTB* (β-actin).

Screening with *Cologuard* involves three steps: (1) Collection of stool by a patient with the *Cologuard* sample collection kit; (2) Laboratory analysis of the sample; (3) Application of an algorithm that produces a positive or negative result for the physician.

The Sample Collection Kit includes Instructions for use, a stool collection container with foldable plastic bracket, a preservative solution, a Protein Sample Tube with stool collection stick and buffer (for the hemoglobin component of *Cologuard*), and a mailing container. Patients collect a sample at home. The sample is sent to a laboratory for processing.

Following sample processing, the assay results are combined into a single composite score. There are five components to the algorithm including a logistic score and 4 decision criteria related to the methylation and mutation assays. The logistic score is derived from a logistic regression based formula that assigns an individual weight to each variable (hemoglobin, 2 methylation genes (*NDRG4*, *BMP3*), 2 groups of mutations in the KRAS gene, and *ACTB*) used in the logistic score evaluation. The specific combination of methylation, mutation, and hemoglobin parameters used in

the logistic score evaluation were chosen because they demonstrated good discriminative ability, stability, sensitivity, and specificity. *ACTB* was chosen to estimate total human DNA in the sample. Four additional decision criteria were also included in the algorithm, one for each methylation and mutation marker, to serve as protection against boundary conditions for DNA markers (but not for the hemoglobin marker, which is measured solely as part of the logistic score). In very rare situations when a single DNA marker has a high value (greater than 99.5% specificity) and other DNA marker values are low, the logistic score may not reach positivity. These boundary conditions were established to protect a true positive sample being called normal using the logistic decision criteria alone. The resulting composite score is compared to a cut-off threshold. A single, positive or negative result is reported; the individual marker results and quantitative score are not presented. The algorithm is presented in **Figure 3** below.

Figure 3: *Cologuard* Algorithm

1) Logistic_Score = X1*Log10(BMP3)+X2*Log10(NDRG4)+X3*Log10(KRAS1)+X4*Log10(KRAS2)+X5*Log10(100/(ACTB ANB+1))+X6*Hemoglobin-2.796044521

Where:

X1=	0.990944982	X4=	-0.392492543
X2=	0.790758688	X5=	1.119602381
X3=	0.428424885	X6=	0.008894634

2) NDRG4_Score = If :Log10 NDRG4 >= Log10(0.112083742) + Log10 ACTB ANB, 10, else 0

3) BMP3_Score = If :Log10 BMP3 >= Log10(0.029294806) + Log10 ACTB ANB, 10, else 0

4) KRAS1_Score = If :Log10 KRAS1 >= Log10(0.043660902) + Log10 ACTB KRAS, 10, else 0

5) KRAS2_Score = If :Log10 KRAS2>= Log10(0.074733554) + Log10 ACTB KRAS, 10, else 0

6) Sum of Scores = Logistic Score + NDRG4_Score + BMP3_Score + KRAS1_Score + KRAS2_Score

7) $$\frac{e^{\text{Sum of Scores}}}{1+ e^{\text{Sum of Scores}}} \times 1000 \; = \; \text{Cologuard Composite Score}$$

8) Cut off positive Cologuard Composite score ≥ 183

Note: Conditions

1. If ACTB ANB or ACTB KRAS < 200 strands Sample invalid (insufficient DNA)
2. If Log10 ACTB KRAS -Log10 ACTB ANB <-0.52 or > 1.04 Sample invalid (ACTB recovery error)
3. If NDRG4, BMP3, KRAS1, KRAS2 <10 strands, set strands to 0: If Marker strands > 300,000 set to 300,000 (range limit of assay)
4. Log10(Marker) = Log10(Marker+1) to avoid log10 (0) condition, for all markers except Log10(100/(ACTB ANB+1))
5. If hemoglobin <lowest calibrator set to 0ng/mL. If hemoglobin >500ng/mL set to 500ng/mL (range of hemoglobin assay)

4.2.1 Principles of Operation

Cologuard sDNA-based testing detects molecular markers of altered DNA present in the cells shed by cancerous and pre-cancerous tumors and polyps into the large bowel. The DNA markers are released from cells that regularly and continuously slough from the lining of the colon into the stool. Through the use of selective enrichment and amplification techniques, *Cologuard* is designed to detect even very small amounts of the DNA markers to identify colorectal cancer or pre-malignant colorectal neoplasia.

As explained above, *Cologuard* is designed to detect three independent families of markers that exhibit an additive association with CRC and pre-malignant colorectal neoplasia. The first DNA family targets epigenetic changes in the form of gene promoter region methylation. The second DNA family targets specific point mutations. The third family of markers is non-DNA based and detects hemoglobin in the fecal sample. As discussed above, the markers that *Cologuard* targets are the *NDRG4* promoter DNA region hypermethylation, *BMP3* promoter DNA region hypermethylation, *KRAS* gene DNA point mutations (7), and fecal hemoglobin. Additionally, *ACTB* is a reference gene used for confirmation and quantitative estimation of the total amount of human DNA present in each sample.

With respect to the methylation markers, the molecular assay component of *Cologuard* is performed to assess the genes *NDRG4* and *BMP3* for aberrant methylation. Aberrant methylation refers to the observation that many genes have elevated methylation in their promoter region in colorectal cancer, whereas the same genes have low levels of methylation in the normal colon epithelial cells. The pattern of methylation relates to which gene promoter regions have hyper-methylation in colorectal cancer compared to the level of methylation in these same promoter regions in normal tissue. The molecular assay component of *Cologuard* is designed to measure methylation in a region of 9 CpG sites in *NDGR4* and 8 CpG sites in *BMP3* promoter regions. In normal colonic epithelia, the degree of methylation in the *NDRG4* promoter region, as determined by the response of the molecular assay component, showed normal epithelial tissue had negligible methylation, while cancer had median values of 14.9%.[57] The molecular assay component detects the extent of methylation in DNA extracted from stool, which represents a mixture of all DNA shed from the colonic lumen. The molecular assay component requires that a high percentage of the CpG sites in the target must be methylated in order to detect the sequence.

With respect to the *KRAS* gene mutations, the mutation assay component of *Cologuard* detects seven common *KRAS* mutations at codons 12 and 13. Mutant *KRAS* gene plays a pivotal role in the development of colorectal cancer in that it is a key activator in colorectal tumorigenesis. As such, *KRAS* mutations provide a valuable marker for the detection of colorectal neoplasms.

The hemoglobin assay component of *Cologuard* is performed to detect the presence of fecal hemoglobin, which can indicate blood loss in the gastrointestinal tract from cancerous tumors or polyps that bleed intermittently into the intestine.[58,59,60] Most cancerous tumors and some polyps

---

[57] Zou H, Allawi H, Cao X, Domanico M, Harrington J, Taylor WR, Yab T, Ahlquist DA, Lidgard G. *Quantification of methylated markers with a multiplex methylation-specific technology*. Clin Chem. 2012; 58(2):375-383.

[58] Greenwald B. *From guiac to immune fecal occult blood tests: the emergence of technology in colorectal cancer screening*. Gastroenterol Nurs. 2005; 28(2):90-6.

[59] Janssens JF. *Faecal occult blood test as a screening test for colorectal cancer*. *Acta Gastro-Enterologica Belgica* 2005; Apr-Jun:244-46.

[60] Levin B, Brooks D, Smith RA, Stone A. *Emerging technologies in screening for colorectal cancer: CT colonography, immunochemical fecal occult blood tests, and stool screening using molecular markers*. CA Cancer J Clin 2003;53:44-55.

bleed, therefore, the presence of fecal hemoglobin is typically used as an indicator of patients who may have unrecognized disease and need follow-up diagnostic testing.[61,62,63] Fecal occult blood testing has been shown to detect CRC at reasonably early stages.[64,65]

A final *Cologuard* composite score (henceforth referred to as "Score") is calculated using a proprietary algorithm that combines a patient's methylation, mutation, and Hemoglobin Assay results. Specifically, the algorithm assigns a weight to each marker assay result and then aggregates these marker results to obtain a composite Score.

If the composite score is less than the clinically confirmed cut-off value, the sample is considered a negative *Cologuard* result. A composite score that is greater than the cut-off value is considered a positive *Cologuard* result.

---

[61] Greenwald B. *From guaiac to immune fecal occult blood tests: the emergence of technology in colorectal cancer screening.* Gastroenterol Nurs. 2005; 28(2):90-6.

[62] Janssens JF. *Faecal occult blood test as a screening test for colorectal cancer.* Acta Gastro-Enterologica Belgica 2005; Apr-Jun:244-46.

[63] Levin B, Brooks D, Smith RA, Stone A. *Emerging technologies in screening for colorectal cancer: CT colonography, immunochemical fecal occult blood tests, and stool screening using molecular markers*. CA Cancer J Clin 2003;53:44-55.

[64] Mandel JS, Church TR, Bond JH, Ederer F, Geisser MS, Mongin SJ, et al. *The effect of fecal occult-blood screening on the incidence of colorectal cancer*. NEJM. 2000;343(22):1603–1607 (demonstrating 20% reduction in CRC incidence).

[65] Mandel JS, Bond JH, Church TR, Snover DC, Bradley GM, Schuman LM, & Ederer F. *Reducing mortality from colorectal cancer by screening for fecal occult blood. Minnesota Colon Cancer Control Study*. NEJM. 1993;328(19):1365–1371 (demonstrating 33% reduction in CRC mortality).

5.0 ANALYTICAL STUDIES

Exact Sciences has conducted extensive analytical testing, both to establish and validate the *Cologuard* algorithm and cut-off threshold for positivity.

First, the company conducted a study utilizing a total of 953 samples. The study dataset consisted of 794 samples determined to be negative for CRC using colonoscopy, 86 samples known to be positive for CRC using colonoscopy and histopathological confirmation, and 73 samples categorized as Advanced Adenomas. These samples were specifically collected and tested as a single cohort for the purpose of establishing the *Cologuard* sample processing algorithm. After the cut-off for the test was established, it was further statistically cross-validated using a dataset comprised of the original 953 samples plus an additional 50 samples. The achieved sensitivity of approximately 98% for cancer and approximately 57% for advanced adenoma met the acceptance criteria.

After the initial cut-off was determined for *Cologuard*, the company verified the robustness of the logistic regression-based predictive algorithm and validated the risk score cut-off using a combination of computer simulations and statistical cross-validation techniques such as Leave-One-Out cross-validation ("LOOCV") and 10-fold cross-validation analyses. Furthermore, various simulations were also performed on the *Cologuard* cut-off study data (n=953) to determine the best estimate of *Cologuard* precision. In addition, final validation of the algorithm and cut-off was performed with the pivotal clinical study described further in Sections 6.0 and 7.0 below.

In addition to the algorithm development and validation work, Exact Sciences has conducted analytical studies of the assay components of *Cologuard.* The analytical testing plan for *Cologuard* was based on relevant FDA guidance and discussions with the agency and was designed to demonstrate the key performance characteristics of *Cologuard*. Consistent with FDA guidance, the following analytical performance characteristics were addressed during analytical method validation of each *Cologuard* component:

Assay	Analytical Performance Characteristics
Cologuard Assay	Precision and Reproducibility (External Lab-to-Lab Reproducibility) Precision of Logistic Risk Score (Algorithm Cut-off and Cross-validation) Robustness Lot-to-Lot Variability
Methylation and Mutation (Molecular Assay) Component	Precision of Biomarkers (External Lab-to-Lab Reproducibility) <u>Sensitivity</u> • Molecular Assay LoD, LoQ, Linearity and Linear Range <u>Specificity</u> • Molecular Assay Specificity to Double *KRAS* Mutations • Molecular Assay Specificity to Wild Type *KRAS* • Molecular Assay Specificity to Partially Methylated Targets • Molecular Assay Specificity to Non-colorectal Cancers and Diseases Interference Substances Carry-over and Cross-contamination
Hemoglobin Assay Component	<u>Sensitivity</u> • Hb Assay LoB, LoD, LoQ, Linearity and Linear Range <u>Specificity</u> • Hb Assay Cross-reactivity and Specificity Interference Substances Carry-over and Cross-contamination

Each test was performed in accordance with a protocol (approved by the company prior to study initiation), which described the success and failure criteria.

The design and results of these tests are described briefly in the table below and further details regarding the analytical testing performed for *Cologuard* can be found in the Summary of Safety and Effectiveness Data provided with this Executive Summary.

Table 1: Analytical Studies

Test	Design	Results/Conclusion
Lab-to-Lab reproducibility	• 7 sample types containing various levels of DNA and hemoglobin • 3 sites, multiple operators, multiple runs	Acceptance criteria met. *Cologuard* results are reproducible across sites and operators
Lot to Lot reproducibility	• 7 sample types containing various levels of DNA and hemoglobin • 3 lots of Cologuard reagents/controls	Acceptance criteria met. *Cologuard* results are reproducible across multiple reagent lots
Molecular Assay Specificity	• Performance in the presence of Double KRAS Mutations, Wild-type KRAS, Partially Methylated Targets, Non-colorectal Cancers and Diseases	Acceptance criteria met. Performance of the assay is not compromised by the presence of these variables
Interfering substances	• Performance in the presence of common substances that could be present in stool materials (e.g. medications, lotions, animal gDNA)	Acceptance criteria met. None of the substances tested interfered with the *Cologuard* molecular or hemoglobin assays
Carry Over and Cross-Contamination	• Sequential runs of high positive and negative samples (carry over) • Checkerboard design, alternating high positive and negative samples, within a run (cross-contamination)	Acceptance criteria met for both molecular and hemoglobin assays, minimal carry over and cross-contamination observed
Hemogobin Assay Cross-reactivity and Specificity	• Ability to detect hemoglobin in specimens heterozygous for Hb S and Hb C (specificity) • Performance in the presence of animal hemoglobin and myoglobin (cross-reactivity)	Acceptance criteria met. Hemoglobin assay can detect hemoglobin from specimens heterozygous for HbS and HbC. Less than 0.1% cross-reactivity with hemoglobin or myoglobin from common edible animals.
Stability Studies	• Stability under standard operating conditions, freeze-thaw stability, real-time stability	Acceptance criteria met. Reagents remained stable under tested conditions

6.0 PIVOTAL STUDY PROTOCOL SUMMARY

6.1 Study Design and Objective

The *Cologuard* pivotal study was a prospective, multi-centered, trial in which the sensitivity (for CRC and AA) and specificity of *Cologuard* in the average-risk screening population were determined. Subjects provided stool samples for both *Cologuard* and FIT (OC FIT-CHEK, Polymedco, Inc.). Results were compared to the results of an optical colonoscopic examination with final confirmation by histopathology for all biopsied and/or excised lesions. Subjects were categorized based on the outcome of colonoscopy and histopathology, as described further below.

The primary objective of this pivotal study was to determine the sensitivity for CRC and specificity of *Cologuard*, using colonoscopy as the reference method. Lesions were confirmed as malignant by histopathologic examination.

The secondary objective of this study was to compare the sensitivity of *Cologuard* to FIT, both with respect to CRC and AA.

The full study protocol can be found in **Appendix B**.

6.2 Study Population

Pivotal clinical study enrollment began June 30, 2011, and a total of 12,776 patients were enrolled at 90 sites. All enrolled subjects have completed participation in the study.

Subjects eligible for enrollment in the study were of both genders between the ages of 50 and 84 years (inclusive), who were at average risk for development of colorectal cancer and asymptomatic for gastrointestinal symptoms warranting diagnostic colonoscopy. Key eligibility criteria follow:

6.2.1 Inclusion Criteria

- Be at average risk for development of colorectal cancer
- Be able and willing to undergo a screening colonoscopy within 90 days of enrollment
- Be 50 to 84 years of age inclusive
- Be able to comprehend, sign, and date the written informed consent document
- Be able and willing to provide stool samples according to written instructions

6.2.2 Exclusion Criteria

- Has any condition which, in the opinion of the Investigator should preclude participation in the study
- Has undergone colonoscopy within the previous nine (9) years
- Has undergone any double-contrast barium enema, virtual (CT-based) colonoscopy, or flexible sigmoidoscopy within the previous five (5) years
- Has a history of colorectal cancer or adenoma
- Has a history of aerodigestive tract cancer
- Has had a positive fecal occult blood test or FIT within the previous six (6) months
- Has had a prior colorectal resection for any reason other than sigmoid diverticular disease
- Has had overt rectal bleeding, e.g., hematochezia or melena, within the previous 30 days. (Blood on toilet paper, after wiping, does not constitute rectal bleeding)

- Has a diagnosis or personal history of any of the following high-risk conditions for colorectal cancer:
    1) Inflammatory bowel disease (IBD) including chronic ulcerative colitis (CUC) and Crohn's disease.
    2) $\geq$ 2 first-degree relatives (*e.g.,* parents, siblings and offspring) who have been diagnosed with colon cancer.
    3) One first-degree relative with CRC diagnosed before the age of 60.
    4) Familial adenomatous polyposis ("FAP", including attenuated FAP)
        a. Hereditary non-polyposis colorectal cancer syndrome ("HNPCC" or "Lynch Syndrome")
        b. Other hereditary cancer syndromes
- Has a family history of:
    1) FAP – Familial Adenomatosis Polyposis Syndrome
    2) HNPCC – Hereditary Non-Polyposis Colorectal Carcinoma
- Participated in any “interventional” clinical study within the previous 30 days in which an experimental treatment is administered or might be administered through a randomized assignment of the subject to one or more study groups

In addition to the enrollment criteria above, subject enrollment was age-weighted toward a slightly older population to increase the point prevalence of CRC in this study; 64% of subjects in the study population were of age 65-84.

A complete list of study sites is provided in **Appendix C**.

6.3 Study Procedures

After providing written informed consent to participate in the study, subjects were provided with a collection kit. Subjects collected a stool at home and returned the sample, which was tested with *Cologuard* and with FIT. The stool samples for *Cologuard* were sent to a central biorepository for subsequent testing at one of three laboratories while the stool samples for FIT were sent to a single laboratory for testing. The laboratory processed FIT samples according to validated procedures and in accordance with ithat test’s instructions for use.

As prescribed in the study protocol, subjects were required to undergo colonoscopy within 90 days of enrollment. Subjects must have completed their stool collection prior to bowel preparation for the scheduled colonoscopy procedure. Subjects were required to perform bowel preparation procedures per the standard of care of the colonoscopy facility. The colonoscopist recorded the quality of bowel preparation as excellent, good, fair, or poor according to the criteria outlined in the protocol. Bowel preparation was required to be at least “fair” in order for the colonoscopy to be acceptable. Subjects with poor bowel preparation were permitted to undergo a repeat colonoscopy as a study subject so long as the second procedure fell within the 90-day window and the Investigator believed it would be in the best interest of the subject. Cecal intubation was required to be documented. Colonoscope withdrawal time was recorded. A completed colonoscopy procedure was defined as reaching the cecum or the junction between the small and large intestine (if the cecum had been resected) or reaching the neo-cecum. A colonoscopy that was incomplete, but that identified a lesion or submitted tissue for histopathological review, was included in the analysis.

As mentioned previously, results were compared to colonoscopy, and histopathology was performed on any biopsied or excised lesions. Histopathology analysis was performed first by a local pathologist to guide treatment decisions for the patient; the histopathology reports were then reviewed by an independent, central pathologist as part of the study, to confirm diagnosis and

categorize subjects for the study. Subjects were categorized as shown in the table below, according to their most clinically significant lesion confirmed on histopathologic analysis (the "index lesion").

Table 2: Categorization of DeeP-C Subjects

Category	Findings
1	CRC, all stages (I-IV)
2	Advanced adenoma, including the following subcategories:
	2.1 Adenoma with carcinoma *in situ*/high grade dysplasia, any size
	2.2 Adenoma, villous growth pattern (≥ 25%), any size
	2.3 Adenoma ≥ 1.0 cm in size
	2.4 Serrated lesion, ≥ 1.0 cm in size
3	1 or 2 adenoma (s), > 5 mm in size, or < 10 mm size, non-advanced
4	≥ 3 adenomas, < 10 mm in size, non-advanced
5	1 or 2 adenoma(s), ≤ 5 mm in size, non-advanced
6	Negative – no neoplastic findings:
	6.1 Negative upon histopathological review (biopsy taken)
	6.2 No findings from colonoscopy, no histopathologic review (no biopsy taken).

The category of a subject's colonoscopic findings determined which of the study analyses the subject was included in or excluded from (e.g., CRC sensitivity analysis included only Category 1 subjects with useable data). Any subjects with lesions identified on colonoscopy but no associated histopathology results (e.g. lesions were not biopsied/excised or samples were lost) were excluded from the analysis, as they could not be categorized per the study protocol. Further discussion of the analysis populations is provided in **Section 7.1.2** below.

Patient management was conducted locally at each investigative site based upon the histopathology results reported by the local pathology lab. When the Central Pathologist histopathology result differed from the local histopathology result, the results were adjudicated by a team of up to three senior pathologists specializing in the diagnosis of gastrointestinal neoplasia to confirm or revise the diagnosis of the referring pathologist (for purposes of the study classification, not patient management). Investigators were notified when the centralized histopathology laboratory assigned a different grade histology result than the local histopathology laboratory.

Subjects and physicians remained blinded to the results of *Cologuard* and FIT throughout the study, and the results were not used in clinical management of the subject. Additionally, *Cologuard* samples were assayed by laboratory technologists blinded to the results of colonoscopy and the comparator FIT test. Similarly, personnel who performed the colonoscopy and produced the resulting report or the personnel who performed the local histopathological review of tissue remained blinded to the testing results.

6.4 Study Endpoints

The primary endpoint was the sensitivity (for CRC) and specificity of *Cologuard*, using colonoscopy with histopathology (when required) as the reference method. The primary analysis required the one-sided 95% lower bound of the sensitivity of *Cologuard* for CRC to exceed 65%. The co-primary analysis used the same methodology to rule out 85% specificity. These preplanned lower bound thresholds developed in consultation with FDA and were based, at least in part, on the best available information in the literature regarding the performance of FIT, as well as physician input regarding the acceptable performance for a new screening test. Considering the potential risk associated with false negatives, *Cologuard* was designed to maximize sensitivity while maintaining a clinically acceptable specificity.

The secondary endpoints of the study compared the performance (sensitivity) of *Cologuard* to FIT, both with respect to cancer and AA. First, *Cologuard* was compared to FIT using a non-inferiority test for CRC sensitivity and using a superiority test for AA sensitivity. If non-inferiority with respect to CRC detection was established, the protocol allowed for penalty-free superiority testing of *Cologuard* compared to FIT.

Adverse events were collected from enrollment until return of the stool collection kit or 90 days post-enrollment, whichever came first. Adverse events ("AEs") caused by or related to the stool collection procedure were not expected because the collection was noninvasive and the test results were not used to make decisions regarding treatment of subjects. The colonoscopy procedure was performed as a routine part of patient care, thus, while adverse events may have been anticipated due to colonoscopy, they were not captured as part of the study.

6.5 Statistical Methods Planned in the Protocol

As mentioned previously, the primary study goals were to establish:

1. The clinically meaningful sensitivity for *Cologuard* according to a one-sided 95% lower bound.

2. The clinically meaningful specificity for *Cologuard* according to a one-sided 95% lower bound.

The secondary study goals were to determine whether *Cologuard* was:

1. non-inferior to the FIT for CRC detection (sensitivity) using a 5% non-inferiority margin according to a one-sided 5% Type I error with 85% power (if non-inferiority with respect to CRC detection was established, the protocol allowed for testing of superiority of *Cologuard* compared to FIT), and

2. superior to FIT for AA detection (sensitivity) according to a one-sided 5% Type I error with 85% power.

Lesions were confirmed as malignant or precancerous by colonoscopy and histopathologic examination. An evaluation of *Cologuard* and FIT with respect to specificity was not performed, due to differing specificity targets of each product.

6.5.1 Sample Size Calculation

Exact confidence interval calculations determined that 42 confirmed CRC cases were required to test the primary endpoint, and paired exact hypothesis tests under various assumptions determined that 49 to 56 confirmed CRC cases were required to test the non-inferiority margin for the difference in sensitivity between *Cologuard* and FIT. Based on projected cancer rates in the average risk

population, an estimated enrollment of approximately 10,500 to 12,000 subjects was required to achieve 80% power for all comparative hypothesis tests. In addition, it was expected that screening the targeted population of this size would yield 525 to 600 AAs, so that the proposed sample size would be sufficient to evaluate both the primary and secondary study objectives.

6.5.2 Study Populations

The results tables presented throughout this report depict the DeeP-C study results according to the Primary Effectiveness Population. The Primary Effectiveness Population included all enrolled subjects, aside from those excluded from the study due to unusable data (e.g., no colonoscopy). This was the primary population used for all analyses, including the assessment of the primary endpoint of the study.

The All Enrolled Subjects Population was used to assess safety, bias, and certain baseline characteristics.

6.5.3 Categorization for Analysis of Sensitivity/Specificity

As prospectively defined in the statistical analysis plan ("SAP"), in determining the sensitivity of *Cologuard* (and FIT, where relevant) for histopathologically confirmed CRC and separately for AAs, the subjects' colonoscopic findings were categorized according to the list provided in the table above and analyzed as follows:

- Category 1. Subjects with colonoscopic findings in category 1 (CRC) were considered to have a positive outcome for the CRC sensitivity calculation.
- Category 2. Subjects with colonoscopic findings in category 2 (AAs) were considered to have a positive outcome for the AA sensitivity calculation and were not included in the CRC analysis.
- Categories 3-6. Subjects with colonoscopic findings in categories 3-6 were treated as negative outcomes.

Per the SAP, specificity was calculated using Categories 3-6. As requested by FDA, Exact Sciences also conducted an analysis of specificity in which AA subjects (Category 2) were included. Exact Sciences believes that the most appropriate analysis of specificity, however, is to treat AA subjects as true positives, because of histopathology considerations, test design, and the clinical importance of AA detection. Specifically, given the cellular expression of AA and CRC, the common histopathology features confirm that an AA has a high probability of progressing to become CRC. Thus, identifying and removing AAs as necessary allows for disease management and potential CRC prevention. Exact Sciences believes this approach is also supported by standard practice on colonoscopy; if a colonoscopist sees a polyp, it will be removed during the colonoscopy procedure since polys and other lesions may have premalignant features from a histopathology perspective. For this reason, *Cologuard* intentionally is designed to identify AA cases, as well as CRC cases, as positive results.

6.6 Additional Analyses

Additional analyses were conducted to assess test performance for various subgroups, by lesion size and lesion location, site size, point of referral site, and other factors.

7.0 PIVOTAL STUDY RESULTS

7.1 Enrollment and Accountability

7.1.1 Enrollment by Site

The study began enrollment on June 30, 2011, and enrolled a total of 12,776 patients at 90 sites, 89 in the United States and 1 in Canada. The Canadian site enrolled about 6.6% of patients and identified 7 CRC cases. The study included enrollment at both primary care sites and colonoscopy centers. Nearly 2,000 subjects were enrolled at primary care sites, although the majority of subjects were enrolled at gastroenterology specialty sites (10,833/12,776, 84.8%).

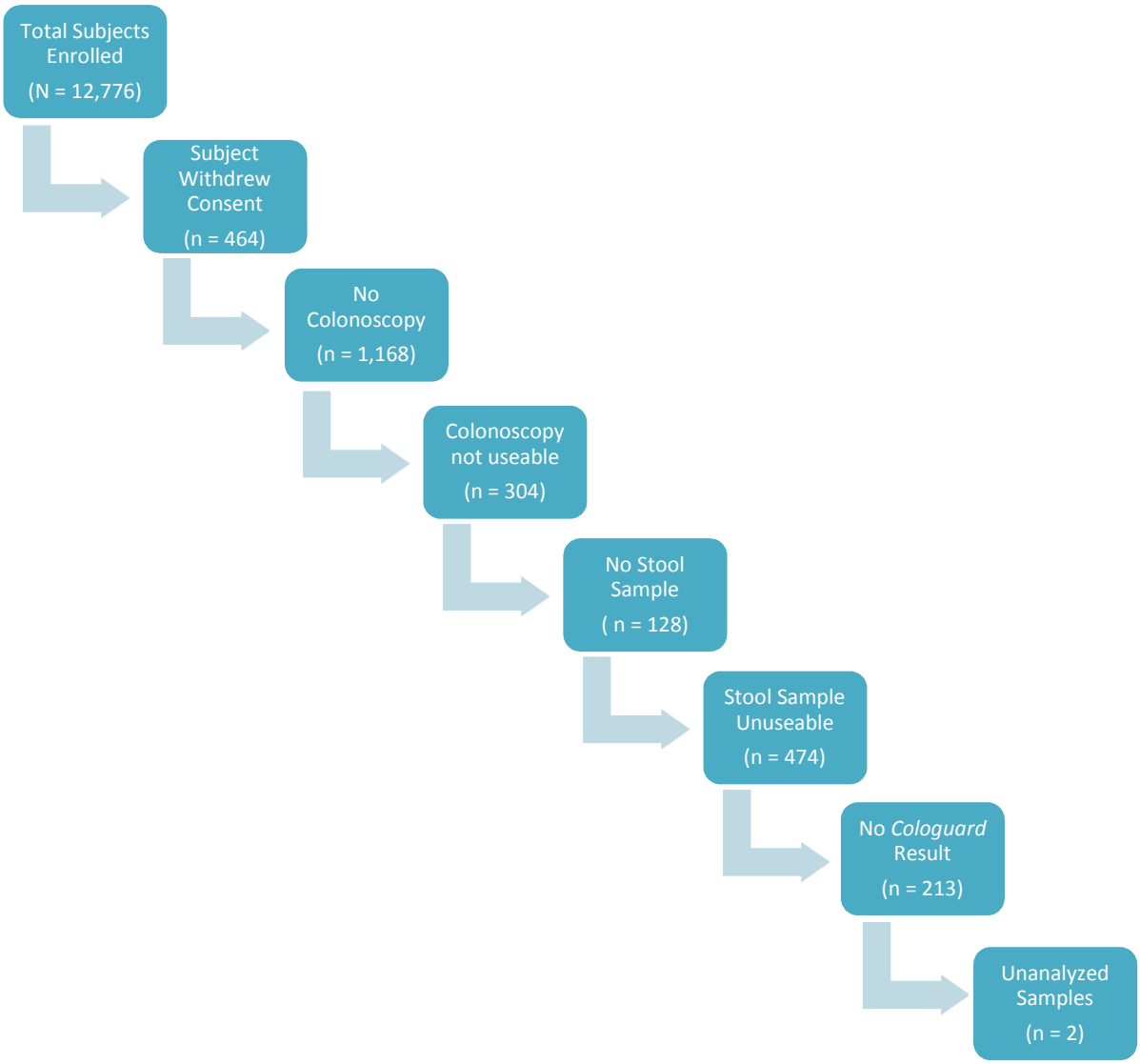
7.1.2 Patient Accountability

A total of 12,776 subjects were enrolled in the Deep-C study, including 76 CRC cases and 822 AA cases. Of these, 10,023 had useable data necessary for the primary endpoint analysis and were included in the Primary Effectiveness Population, including 65 CRC cases and 760 AA cases. In addition, for the secondary endpoint comparison of *Cologuard* with FIT with respect to CRC sensitivity, the population included all subjects in the Primary Effectiveness Population who had a FIT result (N=9,989) ("FIT Secondary Effectiveness" population).

A total of 2,753 subjects were excluded from the primary analysis on the basis of unusable data. Subjects were excluded from all analyses if their colonoscopy, histopathology, or *Cologuard* results were deemed unusable. All subjects who were excluded from the analyses were assessed for bias.

Subjects were excluded for a variety of reasons, including withdrawal of consent, failure to undergo colonoscopy, colonoscopy outside the study window or prior to stool collection (in violation of the protocol), or a lack of colonoscopy findings due to poor bowel prep, incomplete exam, or no cecum inspection.

Figure 4: Subject Accountability – Subjects Excluded from Primary Effectiveness Population



Subjects were excluded from the analysis population in a stepwise fashion. The waterfall diagram in **Figure 4** above provides subject accountability information, showing the number of subjects excluded from the analysis population and the reasons for exclusion.

Subjects in the "Tissue Collected But No Pathology Results" category (shown in **Table 3** below) were cases in which tissue appeared suspicious or indeterminate during colonoscopy and was biopsied, but for whom no histopathological results were available and thus categorization was not possible.

Of the subjects excluded from the analysis population, the majority were excluded because they did not undergo colonoscopy (n = 1,168) or did not have a usable *Cologuard* result (n = 817).

Importantly, the rate of subjects undergoing colonoscopy in the study (90.9%) was still higher than colonoscopy rates in the general CRC screening population, which, historically have been low due to a variety of factors.[66]

While a number of subjects had unusable *Cologuard* results, the majority of these were due to unusable collected stool samples. As shown in **Table 3** below, 73.7% (602/817) of these subjects had either no stool submitted (n = 128) or the stool was unable to be tested (n = 474) (e.g., over weight). Subjects with no stool sample submitted included subjects who failed to collect a stool sample as well as subjects whose samples were lost during transport back to Exact Sciences and who did not collect or provide a second sample. Subjects whose stool samples were unable to be tested included subjects whose samples were submitted with missing components (e.g. no hemoglobin sample provided) or samples that were too large or where the collection kit leaked during transit. For 2 subjects (0.01% of the population), the *Cologuard* result is "missing," because these samples were inadvertently not sent to the laboratories for processing. Importantly, only 1.7% of subjects (213/12,776) had a usable stool sample but were excluded due to an invalid *Cologuard* result.

[66] According to the CDC's most recent estimates, only 65.4% of Americans age 50-75 are adequately screened for CRC (which includes having had a colonoscopy in the past 10 years). *See* Vital Signs: Colorectal Cancer Screening, Incidence, and Mortality --- United States, 2002—2010, *Morbidity and Mortality Weekly Report*, 60(26);884-889, July 8, 2011, *available at*: http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6026a4.htm.

Table 3: Subject Disposition – All Enrolled Subjects

	All Enrolled (N=12776)	Specificity Subset (2-6) (N=10996)	Specificity Subset (3-6) (N=10174)	CRC Subset (N=76)	AA Subset (N=822)	FIT Secondary Effectiveness (N=68)
Enrolled, n	12776	10996	10174	76	822	68
Excluded from Primary Effectiveness, n (%)	2753 (21.5)	1038 (9.4)	976 (9.6)	11 (14.5)	62 (7.5)	3 (4.4)
Subject Withdrew Consent	464 (3.6)	3 (0.0)	3 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Colonoscopy Not Done	1168 (9.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Colonoscopy Not Usable	304 (2.4)	229 (2.1)	225 (2.2)	0 (0.0)	4 (0.5)	0 (0.0)
Colonoscopy >90 days from enrollment	19 (6.3)	19 (8.3)	18 (8.0)	0 (0.0)	1 (25.0)	0 (0.0)
Colonoscopy Before Stool Sample Collection	20 (6.6)	20 (8.7)	17 (7.6)	0 (0.0)	3 (75.0)	0 (0.0)
No Cecum Inspection w/o findings	94 (30.9)	92 (40.2)	92 (40.9)	0 (0.0)	0 (0.0)	0 (0.0)
Incomplete Exam w/o findings	21 (6.9)	19 (8.3)	19 (8.4)	0 (0.0)	0 (0.0)	0 (0.0)
Poor Bowel Prep, no findings	79 (26.0)	79 (34.5)	79 (35.1)	0 (0.0)	0 (0.0)	0 (0.0)
Tissue Collected But No Pathology Results	71 (23.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
No *Cologuard* Result	817 (6.4)	806 (7.3)	748 (7.4)	11 (14.5)	58 (7.1)	3 (4.4)
No Stool Submitted	128 (15.7)	125 (15.5)	114 (15.2)	3 (27.3)	11 (19.0)	0 (0.0)
Stool Not Tested	474 (58.0)	469 (58.2)	434 (58.0)	5 (45.5)	35 (60.3)	0 (0.0)
Tested No Result	213 (26.1)	210 (26.1)	198 (26.5)	3 (27.3)	12 (20.7)	3 (100.0)
Missing Result (Not Tested)	2 (0.2)	2 (0.2)	2 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)

[1] A total of 1704 subjects did not have a case category assigned. 1628 did not have a colonoscopy done, 72 had findings but no pathology results, and 4 had no pathology results and missing findings information.

7.1.3 Study Administration Issues (Protocol Deviations, Minor Administrative Issues)

A breakdown of the protocol deviations that occurred during the study, among the primary effectiveness population, is shown in **Table 4** below.

As described in the protocol, a deviation was defined as any change, divergence, or departure from the study design or procedures of the protocol that was under the Investigator's control and not been approved by the IRB. Most protocol deviations in the study were minor deviations, such as administrative or clerical errors. Certain types of deviations, such as a missing colonoscopy, resulted in the subject being excluded from the analysis population. Those types of deviations are adressed above in the **Patient Accountability** discussion above.

Beyond the deviations that resulted in exclusion of the subject from analysis, the table below summarizes key protocol deviations that occurred during the DeeP-C Study. Administrative or clerical errors during the consent process (e.g. failure to initial one page of a multi-page consent document, failure to date one page of multi-page document) were reported for 156 subjects (1.5%) and were ultimately resolved. In no case was an informed consent form completely missing or unsigned for a study subject. None of the consent deviations were deemed sufficient to require exclusion. Additionally, 96 subjects (1.0%) did not meet the eligibility criteria for the study due to age, colonoscopy in the past 9 years, or because they were considered "high risk" for CRC. However, because the percentage of subjects deemed to be ineligible was small and similar patients may be included in the population that receives the test in clinical practice, these subjects were included in the analysis population. An analysis excluding the 2 subjects who were included in the Primary Effectiveness Population despite being < 50 years of age is presented in **Section 7.3** below.

Table 4: Protocol Deviations, by Type

		All Enrolled
Not consented properly	Missing or inadequate consent requiring exclusion	0
	Administrative consent error	156
Did not meet inclusion/exclusion criteria[1]	Subject with colonoscopy within past 9 years	29
	Age outside range	3
	High risk	17
	Medical condition precluding inclusion	1
	Double-contrast barium enema, virtual colonoscopy, or flexible sigmoidoscopy within the past 5 years	4
	History of CRC or adenoma	16
	History of aerodigestive tract cancer	7
	Positive FOBT or FIT within the past 6 months	8
	Prior colorectal resection for reason other than sigmoid diverticular disease	7
	Overt rectal bleeding, past 30 days	4
	All eligibility deviations	96

[1] Some subjects had multiple eligibility deviations entered. Subject is shown in most clinically notable category.

7.2 Demographics and Baseline Characteristics

The baseline demographic characteristics for the Primary Effectiveness Population are presented in **Table 5** below. As shown in the table, the average age of subjects was 64.2 years old, and there were a higher percentage of female subjects as compared with male subjects. The majority of subjects were White, with the next most common racial group being Black or African American subjects. Nearly 10% of subjects were Hispanic or Latino.

It should be noted that two subjects were included in the analysis population who were enrolled shortly before their 50th birthdays, and therefore did not meet the inclusion criteria for the study. Similarly, one 44-year-old subject was included who would not have met the inclusion criteria. Each of these subjects was negative on both the *Cologuard* and FIT tests and was negative by colonoscopy. They were included to provide the boadest assessment of performance. Because they were true negatives for purposes of study data analysis, their inclusion does not impact any sensitivity analysis. Due to the large number of negative subjects in the analysis population, inclusion of these two subjects had no notable impact on the calculation of specificity.

Notably, the DeeP-C population mirrored the general CRC screening population in the United States, as described in the literature.[67] The age distribution of subjects in DeeP-C was similar to the general CRC screening population, with the largest proportion of subjects between 65 and 74 years old, and the next highest proportion of subjects between the ages of 75 and 84. Of note, subject enrollment in DeeP-C was age-weighted toward a slightly older population to increase the point prevalence of CRC in the study; 64% of subjects in the study population were of age 65-84.

The gender distribution of subjects in DeeP-C (53.7% female, 46.3% male) was almost identical to the U.S. CRC screening population (53.6% female and 46.4% male). Additionally, the distribution of DeeP-C subjects by race and ethnicity was similar to that of the U.S. CRC screening population. Caucasian/White subjects made up 84.1% of the DeeP-C population, compared with 81.95% of the U.S. screening population, while Black/African-Americans made up 10.7% of DeeP-C subjects, compared with 12.65% of the general CRC screening population. DeeP-C had a larger percentage of Hispanic/Latino subjects (9.9%), compared with the U.S. CRC screening population (2.05%).

Table 5: Baseline Demographics – Primary Effectiveness Subjects

Parameter Statistic	**All Enrolled (N=10023)**	**CRC Subset (N=65)**	**AA Subset (N=760)**
Age (years) at Screening			
N	10023	65	760
Mean (SD)	64.2 (8.42)	70.2 (7.92)	65.4 (7.93)
Median	66	70	66
Min, Max	44, 84	50, 84	50, 84
Gender, n (%)			
Male	4645 (46.3)	34 (52.3)	450 (59.2)
Female	5378 (53.7)	31 (47.7)	310 (40.8)

[67] Schenck AP, Peacock SC, Klabunde CN, Lapin P, Coan JF, Brown ML. (2009) Trends in Colorectal Cancer Test Use in the Medicare Population, 1998–2005. *Am J Prev Med* 37(1).

Parameter Statistic	All Enrolled (N=10023)	CRC Subset (N=65)	AA Subset (N=760)
Race, n (%)			
White	8422 (84.1)	55 (84.6)	641 (84.5)
Black or African American	1071 (10.7)	8 (12.3)	85 (11.2)
Asian	259 (2.6)	1 (1.5)	13 (1.7)
American Indian or Alaska Native	36 (0.4)	0 (0.0)	4 (0.5)
Native Hawaiian or Other Pacific Islander	23 (0.2)	0 (0.0)	0 (0.0)
Other	206 (2.1)	1 (1.5)	16 (2.1)
Missing	6	0	1
Ethnicity, n (%)			
Hispanic or Latino	991 (9.9)	9 (13.8)	59 (7.8)
Not Hispanic or Latino	9028 (90.1)	56 (86.2)	700 (92.2)
Missing	4	0	1
BMI (kg/m2) at Baseline			
N	10015	65	760
Mean (SD)	28.83 (5.836)	27.55 (4.861)	29.67 (6.068)
Median	28.0	26.8	29.0
Min, Max	13.3, 68.2	19.3, 42.4	16.3, 59.9
Smoking History, n (%)			
Never Smoked	5531 (55.2)	33 (50.8)	341 (44.9)
Former Smoker	3589 (35.8)	25 (38.5)	285 (37.5)
Current Smoker	903 (9.0)	7 (10.8)	134 (17.6)
If Former or Current Smoker, Daily Use, n (%)			
<1/2 Pack Per Day	2162 (48.3)	8 (25.0)	184 (44.0)
1 Pack Per Day	1585 (35.4)	16 (50.0)	151 (36.1)
>1 Pack Per Day	732 (16.3)	8 (25.0)	83 (19.9)
Missing	13	0	1
If Former or Current Smoker, # Years Smoking			
N	4480	32	419
Mean (SD)	21.82 (14.733)	28.47 (13.488)	27.93 (15.959)
Median	20.0	29.0	30.0
Min, Max	0.0, 70.0	1.0, 60.0	1.0, 65.0

Exact Sciences also compared the demographics of subjects enrolled at primary care (point of referral) sites (Principal Investigator is a primary care physician) and gastroenterology specialty sites

(Principal Investigator is gastroenterology specialist). In general there were no notable differences in the populations enrolled at these two types of sites.

In addition, as only one study site was located outside the U.S. (Site 122, in Canada) the site was evaluated for poolability relative to all other study sites using multiple approaches. From a demographics perspective, this site did not include African-Americans or subjects of Hispanic or Latin origin, but was generally representative from an age and gender perspective. From an outcomes perspective, analyses were conducted to assess the percents positive between the two site types. Based on these analyses, it was determined that this Canadian site was consistent (poolable) with the U.S. sites.

7.3 Primary Effectiveness Evaluations (Sensitivity/Specificity)

Results from the DeeP-C study demonstrated that *Cologuard* successfully met the primary endpoint of the study, establishing a clinically meaningful sensitivity and specificity substantially exceeding the pre-specified criteria for study success.

As shown in **Table 6** below, sensitivity of *Cologuard* for CRC was 92.3% (60/65) with a one-sided 95% confidence interval lower bound of 84.5%, well above the pre-specified threshold of 65%. Thus, the study was a success with respect to sensitivity for CRC.

Table 6: Overall Sensitivity for CRC – Primary Effectiveness Subjects

	Valid *Cologuard* (N=65) Positive Result
Case Category, n/N (%)	
1: CRC Stages 1-4	92.3% (60/65)
Sensitivity Based on Category 1: Primary (one-sided 95% lower bound)	92.3% (>84.5%)
Sensitivity Based on Category 1: Supportive (two-sided 95% % lower bound)	92.3% (>83.0%)

[1] Percentages based on valid test results within a category.
[2] Lower bounds calculated using an exact one-sided binomial test.

As shown in **Table 7** below, the specificity of *Cologuard* was 86.6%, with a one-sided 95% confidence interval lower bound of 86.0%, above the pre-specified threshold of 85%. Thus, the study was a success with respect to specificity.

Therefore, *Cologuard* met both primary endpoint analysis thresholds and, as a result, the DeeP-C study was declared a success.

Table 7: Overall Specificity – Primary Effectiveness Subjects

	Valid *Cologuard* (N=9198) Negative Result
Case Category, n/N (%)	
3: 1-2 Adenomas 5-<10 mm	607/749 (81.0%)
4: ≥3 Adenomas <10 mm, Non-advanced	302/419 (72.1%)
5: 1-2 Adenomas ≤5 mm, Non-advanced	1496/1735 (86.2%)
6.1: Negative upon histopathological review	1543/1821 (84.7%)
6.2: No findings on colonoscopy, no histopathological review	4019/4474 (89.8%)
Specificity Based on Categories 3-6: Primary (one-sided 95% lower bound)	86.6% (>86.0%)
Specificity Based on Categories 3-6: Supportive (one-sided 97. 5% lower bound)	86.6% (>85.9%)

[1] Percentages based on valid test results within a category.
[2] Lower bounds calculates using an exact one-sided binomial test.
[3] Two 49-year-old and one 44-year-old true negative subjects were included in the analysis population, although they would not be included in the intended user population.

7.4 Alternative Specificity Analysis

Specificity, as presented in the primary endpoint section above, was calculated, consistent with the study protocol and SAP, based on Categories 3 through 6 (smaller, non-advanced adenomas and negatives with no neoplastic findings). Given that *Cologuard* is designed to detect AA, all AA cases were treated as true positives and excluded from the analysis of specificity. As AAs are considered to be the clinically relevant precursors of CRC disease and have been referred to as "the most valid neoplastic surrogate marker for present and future colorectal cancer risk," Exact Sciences believes the presence of AA indicates the presence of disease and, as such, these cases should be excluded in the specificity analysis.[68] Notably, subjects falling into these categories would typically all receive excisional surgery as part of their treatment plan.

Nonetheless, per FDA's request, Exact Sciences also calculated specificity for *Cologuard* assuming all non-CRC findings (including AA) were true negatives, such that all subjects in Categories 2-6 were included in the specificity analysis. Specificity of *Cologuard* as calculated per this method declined only slightly to 84.4%, as shown in **Table 8** below..

[68]Brenner H, Hoffmeister M, Stegmaier C, Brenner G, Altenhofen L, and Haug U. (2007) Risk of progression of advanced adenomas to colorectal cancer by age and sex: estimates based on 840 149 screening colonoscopies. *Gut* 56(11): 1585–1589; Winawer SJ, Zauber AG. (2002) The advanced adenoma as the primary target of screening. *Gastrointest Endosc Clin N Am.* 12(1):1-9.

Table 8: Alternative Specificity Analysis – Primary Effectiveness Subjects

	Valid *Cologuard* (N=9958) Negative Result
Case Category, n/N (%)	
2.1: Adenoma with carcinoma *in situ*/high grade dysplasia	30.8% (12/39)
2.2: Adenoma, villous growth pattern ≥ 25%	55.9% (143/256)
2.3: Adenoma ≥10 mm	61.9% (226/365)
2.4: Serrated Lesion ≥10 mm	57.0% (57/100)
3: 1-2 Adenomas 5-<10 mm	81.0% (607/749)
4: ≥3 Adenomas <10 mm, Non-advanced	72.1% (302/419)
5: 1-2 Adenomas ≤5 mm, Non-advanced	86.2% (1496/1735)
6.1: Negative upon histopathological review	84.7% (1543/1821)
6.2: No findings from colonoscopy, no histopathological review	89.8% (4019/4474)
Specificity Based on Categories 2-6: Supportive (one-sided 95% lower bound)	84.4% (>83.8%)
Specificity Based on Categories 2-6: Supportive (one-sided 97.5% lower bound)	84.4% (>83.7%)

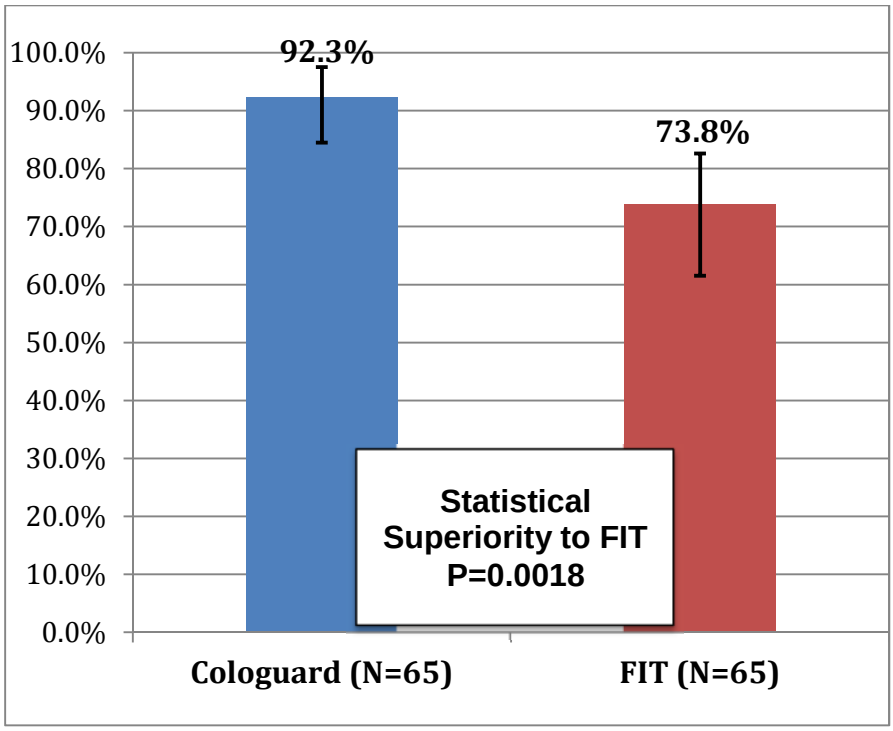
[1] Percentages based on valid test results within a category.
[2] Lower bounds calculated using an exact one-sided binomial test

7.5 Secondary Effectiveness Evaluations

The secondary endpoint analysis compared *Cologuard* to FIT. Only subjects with a valid *Cologuard* and FIT test could be included in this secondary effectiveness analysis. As such, the total number of subjects in the analysis differ from those included in the Primary Effectiveness Population by the number of AA subjects (n = 3) who were missing a valid FIT result.

The secondary endpoint analysis demonstrates that *Cologuard* is statistically superior to FIT with respect to detection of both CRC and AA. As shown in **Figure 5** below, sensitivity of *Cologuard* for CRC was 92.3% (60/65), compared with 73.8% (48/65) for FIT (p=0.0018). Further, as shown in Figure 7 below, sensitivity of *Cologuard* for AA was 42.4% (321/757) compared with 23.8% (180/757) for FIT (p<0.0001).

Figure 5: Secondary Endpoint - CRC Comparison to FIT



Per the protocol specified analysis, the results demonstrated that *Cologuard* was non-inferior to FIT for CRC. The lower bound of the one-sided confidence interval for the difference in sensitivities of Cologuard and FIT was 0.080, substantially exceeding the protocol-specified non-inferiority threshold of -0.05. The protocol also provided for superiority analysis for CRC detection. *Cologuard* demonstrated superiority over FIT for CRC sensitivity with a one-sided p-value (p=0.0018) well below the one-sided p <0.025 threshold for superiority.

As shown in the 2x2 table below, *Cologuard* correctly captured 60 of the 65 total CRC cases identified by colonoscopy (92.3%). Meanwhile, FIT captured only 48 of the 65 CRC cases identified by colonoscopy (73.8%). Notably, FIT identified only a single cancer that was not identified by *Cologuard*. *Cologuard*, meanwhile, identified 13 cancers that were missed by FIT.

*Cologuard w*as also found to be superior to FIT for AA detection. In this analysis, an exact McNemar's comparison test was performed; a one-sided p-value <0.025 was required to achieve superiority.

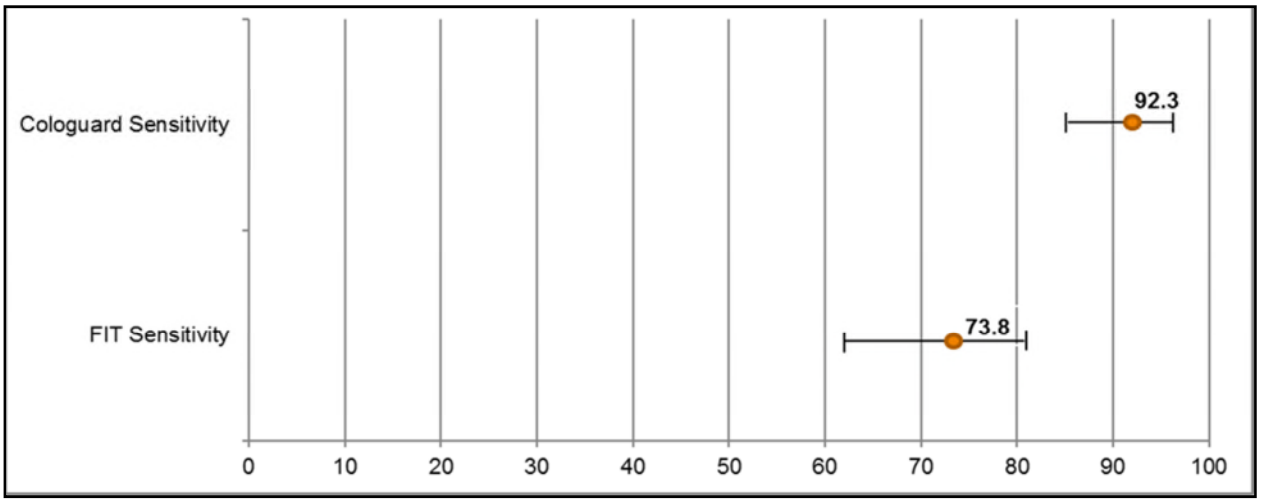
Table 9: Sensitivity Non-Inferiority Test – CRC Subset (Category 1)

		FIT Outcome			
	***Cologuard* Outcome**	**Negative**	**Positive**	**Totals**	**McNemar test p-value**
Category 1	**Negative, n (%)**	4 (80.0)	1 (20.0)	5	0.001831055
	Positive, n (%)	13 (21.7)	47 (78.3)	60	
	Totals	17	48	65	

[1] p-value is from a McNemar paired comparison test of the discordant pairs.
[2] One-sided 5% lower bound on the discordant pair difference for Category 1 to rule out a 5% non-inferiority margin = 9.5% > -5%.
[3] One-sided 2.5% lower bound on the discordant pair difference for Category 1 to rule out a 5% non-inferiority margin = 7.3% > -5%.

Sensitivity of *Cologuard* for CRC so far exceeded that of FIT that the two-sided 95% confidence intervals for the sensitivity estimates of the two tests did not overlap. As shown in **Figure 6** below, the lower bound of the 95% confidence interval for *Cologuard* sensitivity (84.5%) was still higher than the upper bound of the 95% confidence interval for FIT sensitivity for CRC (82.6%).

Figure 6: CRC Sensitivity



The secondary endpoint analyses also found *Cologuard* to be superior to FIT with respect to AA sensitivity. As shown in **Figure 7** and **Table 10** below, *o*verall *Cologuard* sensitivity for AA was 42.4% compared with 23.8% for FIT. *Cologuard* successfully demonstrated superiority over FIT for AA sensitivity with a one-sided p-value ($p < 0.0001$) well below the one-sided $p < 0.025$ threshold for superiority.

Figure 7: Secondary Endpoint - AA Comparison to FIT

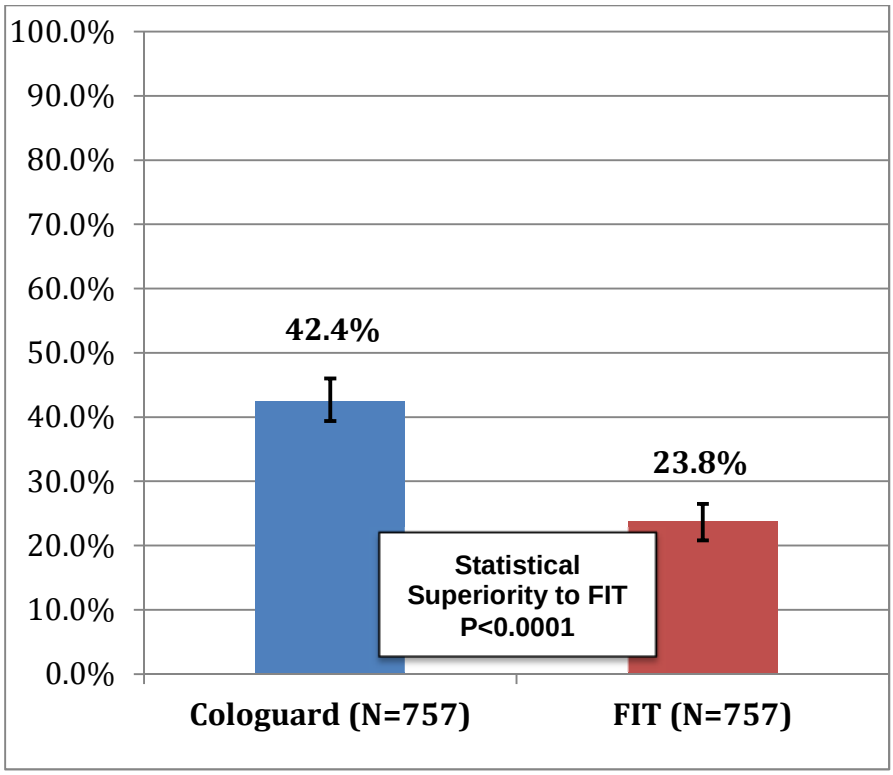


Table 10: Sensitivity – Secondary Effectiveness Subjects, AA Subset (Category 2)

	Valid *Cologuard* (N=757) Positive Result	Valid FIT (N=757) Positive Result
Case Category, n/N (%)		
2.1: Adenoma with carcinoma *in situ*/high grade dysplasia	27/39 (69.2%)	18/39 (46.2%)
2.2: Adenoma, villous growth pattern ≥ 25%	113/256 (44.1%)	66/256 (25.8%)
2.3: Adenoma ≥10 mm	139/363 (38.3%)	91/363 (25.1%)
2.4: Serrated Lesion ≥10 mm	42/99 (42.4%)	5/99 (5.1%)
Sensitivity Based on Category 2: Primary (one-sided 95% lower bound)	42.4% (>39.4%)	23.8% (>21.2%)
Sensitivity Based on Category 2: Supportive (one-sided 97.5 lower bound)	42.4% (>38.9%)	23.8% (>20.8%)

[1] Percentages based on valid test results within a category.
[2] Lower bounds calculated using an exact one-sided binomial test.

Notably, as shown in **Table 11**, FIT identified only 29 AA cases that were not captured by *Cologuard*, while *Cologuard* identified 170 AA cases that were not positive on the FIT test.

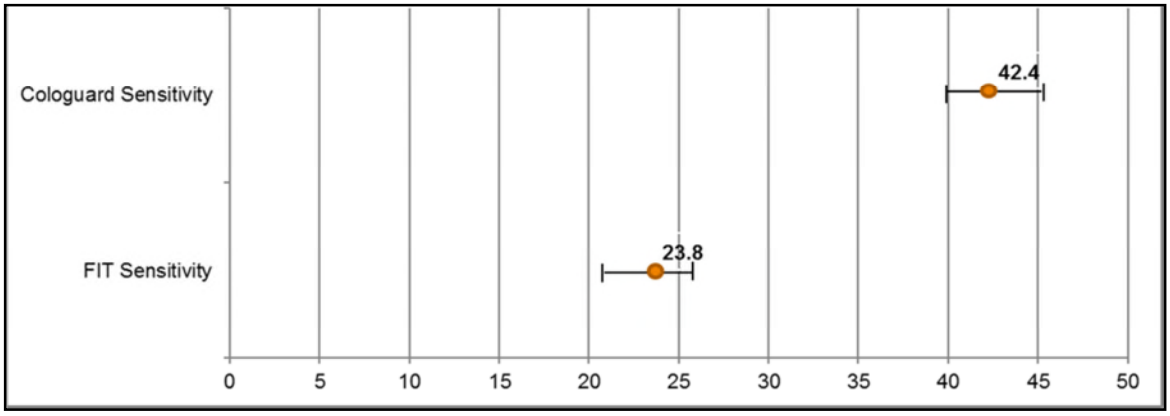
Table 11: Sensitivity Superiority Test – AA Subset (Category 2)

		FIT Outcome			
	***Cologuard* Outcome**	**Negative**	**Positive**	**Totals**	**McNemar test p-value**
Category 2	**Negative, n (%)**	407 (93.3)	29 (6.7)	436	<0.000000001
	Positive, n (%)	170 (53.0)	151 (47.0)	321	
	Totals	577	180	757	

[1] p-value is from a McNemar paired comparison test of the discordant pairs.
[2] One-sided 5% lower bound on the discordant pair difference for Category 2 to rule out a 5% non-inferiority margin = 15.8% > -5%.
[3] One-sided 2.5% lower bound on the discordant pair difference for Category 2 to rule out a 5% non-inferiority margin = 15.3% > -5%.

Again, sensitivity of *Cologuard* for AA so far exceeded that of FIT that the two-sided 95% confidence intervals for the sensitivity estimates of the two tests did not overlap. As shown in **Figure 8** below, the lower bound of the 95% confidence interval for *Cologuard* sensitivity (39.4%) was well above the upper bound of the 95% confidence interval for FIT sensitivity for AA (26.5%).

Figure 8: AA Sensitivity



The numerical advantage in sensitivity for *Cologuard* was observed across all sub-categories of AA, as shown in **Table 10** above. For example, sensitivity for adenoma with carcinoma *in situ*/high grade dysplasia (Category 2.1) was 69.2% for *Cologuard.* This represents a significant sensitivity advantage over FIT (46.2% for adenoma with carcinoma *in situ*/high grade dysplasia). Importantly, *Cologuard* provides a significant advantage in sensitivity for serrated lesions, which historically have been difficult to capture with FIT, because they do not bleed. The serrated polyp pathway, which is distinct from the conventional adenoma-carcinoma pathway, is increasingly recognized as an important pathway in the development of CRC.[69] It has been estimated that 10-20% of CRCs evolve from serrated polyps. As shown in the table, *Cologuard* sensitivity for serrated lesions was 43.0%, compared with 5.1% for FIT.

[69] Mäkinen MJ (2007). "Colorectal serrated adenocarcinoma". *Histopath* 50 (1): 131–50.

Table 12 below presents direct comparisons of *Cologuard* and FIT, in terms of positive/negative agreement across all categories. As shown in the table, for CRC cases, in 20.0% of cases, *Cologuard* yielded a positive result, while FIT was negative. In only one case did FIT identify a CRC case that was not identified by *Cologuard*. For AA cases, in a higher percentage of cases (22.5%), *Cologuard* successfully identified an AA case while FIT did not. In only 3.8% of cases did FIT identify an AA that was missed by *Cologuard*, confirming that *Cologuard* provides a significant advantage over FIT with respect to detection of AA.

Table 12: *Cologuard* and FIT – Primary Effectiveness Subjects (Both Tests Valid)

	Categories 1-6					
Joint Outcomes	**Category 1 (CRC)**	**Category 2 (AA)**	**Category 3 (1-2 >5 mm)**	**Category 4 (≥3 <10 mm)**	**Category 5 (1-2 ≤5 mm)**	**Category 6 (6.1 or 6.2)**
EXACT Negative \ FIT Negative, n (%)	4 (6.2)	407 (53.8)	584 (78.5)	287 (68.5)	1461 (84.5)	5455 (86.9)
EXACT Negative \ FIT Positive, n (%)	1 (1.5)	29 (3.8)	18 (2.4)	15 (3.6)	30 (1.7)	86 (1.4)
EXACT Positive \ FIT Negative, n (%)	13 (20.0)	170 (22.5)	96 (12.9)	72 (17.2)	173 (10.0)	567 (9.0)
EXACT Positive \ FIT Positive, n (%)	47 (72.3)	151 (19.9)	46 (6.2)	45 (10.7)	66 (3.8)	166 (2.6)

The incremental benefit of *Cologuard* over FIT was calculated and is presented in detail in **Table 13** below. As shown in the table, *Cologuard* yielded a 20.0 percentage point incremental benefit over FIT for CRC detection (95% lower bound = 11.1 percentage points). *Cologuard* had a 22.5 percentage point additional benefit as compared with FIT for AA detection (95% lower bound = 19.5 percentage points).

Table 13: *Cologuard* Incremental Value
Primary Effectiveness Subjects with Both Tests Valid

		Category 1 (CRC)	Category 2 (AA)
CRC	EXACT Positive for Category 1	60 (92.3%)	
	95% Lower Bound	83.0%	
	FIT Positive for Category 1	48 (73.8%)	
	95% Lower Bound	61.5%	
AA	EXACT Positive for Category 2		321 (42.4%)
	95% Lower Bound		38.9%
	FIT Positive for Category 2		180 (23.8%)
	95% Lower Bound		20.8%
EXACT Incremental Benefit for CRC	EXACT Positive and FIT Negative	13 (20.0)%	
	95% Lower Bound	11.1%	
EXACT Incremental Benefit for AA	EXACT Positive and FIT Negative		170 (22.5)%
	95% Lower Bound		19.5%

[1] 95% lower bound calculated using an exact binomial distribution.

7.6 Alternative Sensitivity Analysis

Exact Sciences also performed an additional analysis to provide sensitivity for the combined categories of CRC and advanced adenomas (advanced neoplasia). The combined sensitivity for CRC and AA subjects is provided in **Table 14** below. As shown in the table, *Cologuard* sensitivity is 46.3% while FIT sensitivity is 27.7%. Even under this analysis, *Cologuard* maintained a nearly 20% absolute advantage in sensitivity over FIT.

Table 14: Sensitivity for Advanced Neoplasia (CRC + AA)

	***Cologuard* N=822**	**PolyMedco FIT N=822**
	Sensitivity	Sensitivity
Category 1 Only	92.3% (60/65)	73.8% (48/65)
Categories 1-2	46.4% (381/822)	27.7% (228/822)

7.7 Specificity Comparison to FIT

With respect to specificity, a formal comparison to FIT was not planned in the study protocol as the two tests are designed to have different specificities. However, the company compared the number

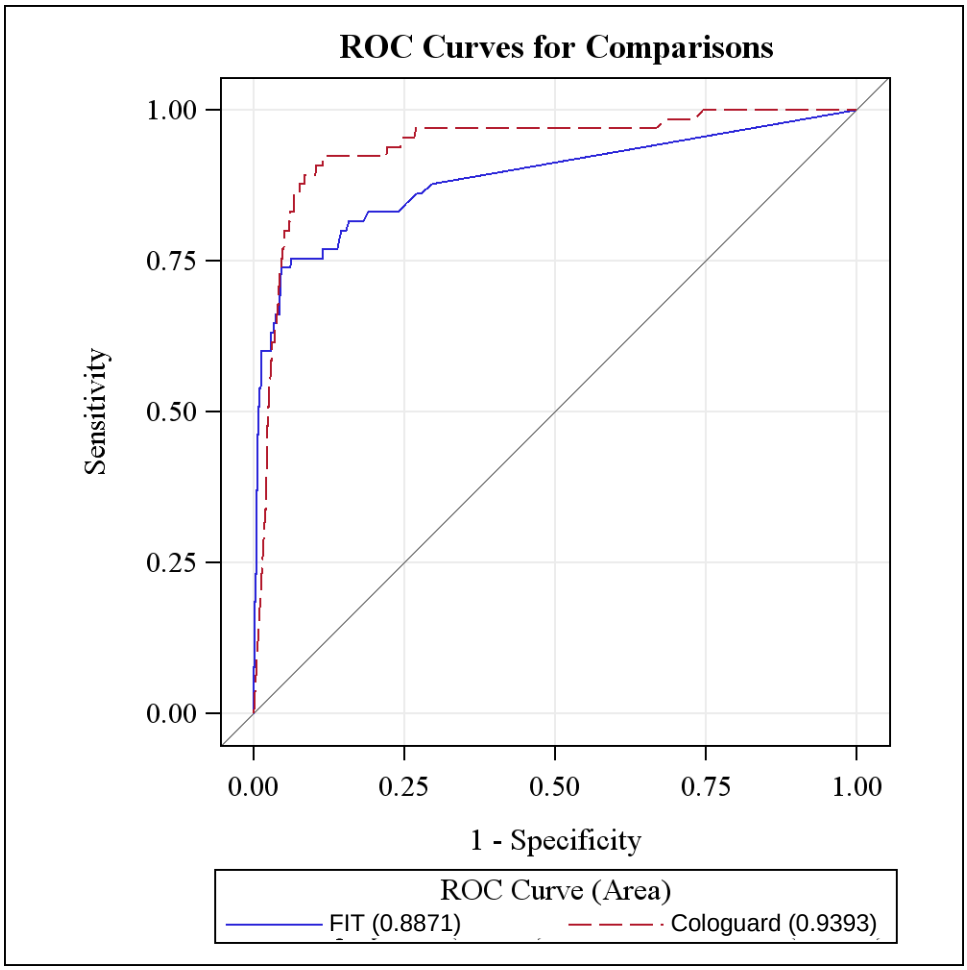
of true negatives captured by *Cologuard* out of those identified by colonoscopy (7,936/9,167, 86.6%), to those captured by FIT, as shown in the 2x2 table below. FIT captured more true negatives, (8,695/9,167 94.9%), but the FIT test by design has a higher specificity, and consequently, significantly lower sensitivity than *Cologuard.*

Table 15: Specificity – Specificity Subset (Categories 3-6)

		FIT Outcome		
	***Cologuard* Outcome**	**Negative**	**Positive**	**Totals**
Categories 3-6	**Negative, n (%)**	7787 (98.1)%	149 (1.9)%	7936
	Positive, n (%)	908 (73.8)%	323 (26.2)%	1231
	Totals	8695	472	9167

Exact Sciences also conducted an Receiver Operating Characteristic curve ("ROC curve") analysis to evaluate whether the trade-off between sensitivity and specificity for *Cologuard* is appropriate. The results, shown in **Figure 9** below, demonstate that a randomly selected CRC patient would be 93.9% more likely to have a higher test value than a negative patient. By comparison, a randomly selected CRC patient would be 88.7% more likely to have a higher FIT test value than a negative patient. The two sided p-value for the difference was statistically significant (p=0.0372).

Figure 9: CRC Sensitivity Using Categories 3-6 for Specificity: *Cologuard* vs FIT – Secondary Effectiveness Population



7.8 Additional Analyses of Effectiveness

7.8.1 Likelihood Ratios and Predictive Values

In addition to sensitivity and specificity, the positive and negative likelihood ratios for *Cologuard* were calculated from the study data. Results demonstrated a positive likelihood ratio ("PLR") of 6.874 for CRC, indicating that a person with CRC would be 6.9 times more likely to have a positive *Cologuard* results than someone without CRC. Results for AA showed a PLR of 3.158, again demonstrating that the test is informative.

Table 16: Positive Likelihood Ratio – Primary Effectiveness Subjects

	Category 1 (CRC) vs Categories 3-6	Category 2 (AA) vs Categories 3-6
Positive Likelihood Ratio (PLR)		
Sensitivity	92.3	42.4
1-Specificity	13.4	13.4
PLR	6.874	3.158
95% Confidence Interval	(6.299, 7.501)	(2.863, 3.483)

The negative likelihood ratio ("NLR") for CRC was 0.089, indicating that someone without CRC is approximately 11 times (1/0.089) more likely to test negative on *Cologuard* compared to someone with CRC. Results for AA showed a NLR of 0.665, again demonstrating that the test is informative.

Table 17: Negative Likelihood Ratio – Primary Effectiveness Subjects

	Category 1 (CRC) vs Categories 3-6	Category 2 (AA) vs Categories 3-6
Negative Likelihood Ratio (NLR)		
1-Sensitivity	7.7	57.6
Specificity	86.6	86.6
NLR	0.089	0.665
95% Confidence Interval	(0.038, 0.206)	(0.626, 0.708)

Analysis was also performed to calculate the positive and negative predictive values ("PPV" and "NPV") for *Cologuard*. As with any CRC screening test, the PPV is impacted by the very low prevalence of CRC in the general population. The PPV was calculated to be 3.72% (60/1613) for CRC and 19.86% (322/1613) for AA, which is in the range of estimates for other CRC screening tests. For example, the PPV of the fecal occult blood test (FOBT) in a much smaller population of symptomatic patients was estimated to be 7.3% for CRC.[70] Meanwhile, the NPV was 94.73%.

Table 18: Positive Predictive Value – Primary Effectiveness Subjects

Cologuard	**Category 1 (CRC)**	**Category 2 (AA)**	**Categories 3-6**
Negative	0.06, 0.02-0.14 (5/8410)	5.21, 4.74-5.71 (438/8410)	94.73, 94.23-95.20 (7967/8410)
Positive	3.72, 2.85- 4.76 (60/1613)	19.96, 18.0-22.0 (322/1613)	76.32, 74.16-78.37 (1231/1613)

[70] Niv Y, Sperber AD. (1995) Sensitivity, specificity, and predictive value of fecal occult blood testing (Hemoccult II) for colorectal neoplasia in symptomatic patients: a prospective study with total colonoscopy. *Am J Gastroenterol* 90(11):1974-7.

*2-Sided 95% CIs

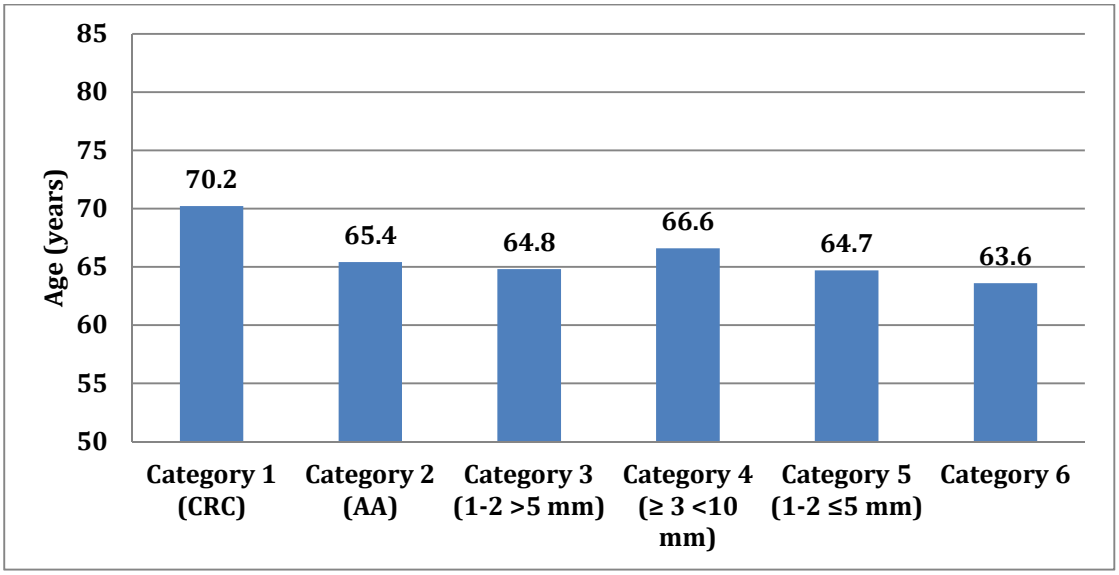
7.8.2 Subgroup Analyses

The DeeP-C study was not powered to assess differences in *Cologuard* performance by subgroups, nonetheless, consistent with the protocol and statistical analysis plan, Exact Sciences evaluated *Cologuard* sensitivity and specificity by various demographic characteristics, type of study site, and lesion location and size. These additional presentations of the data are described in the sections below.

7.8.2.1 Results by Age

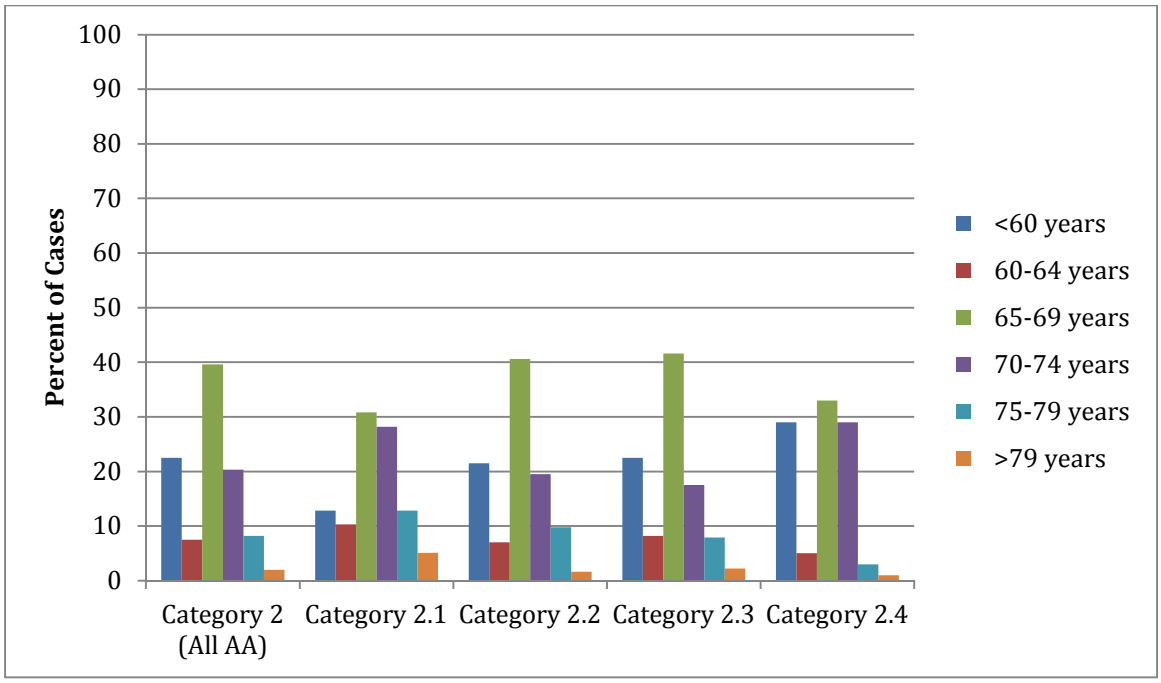
Results for *Cologuard* and FIT are presented by age in the tables that follow. Mean and median age, by category, is presented in **Figure 10** below. As shown in the table, CRC cases were generally older than the other study subjects, as can be expected given the natural history of CRC progression.

Figure 10: Mean Age, by Category – Primary Effectiveness Subjects



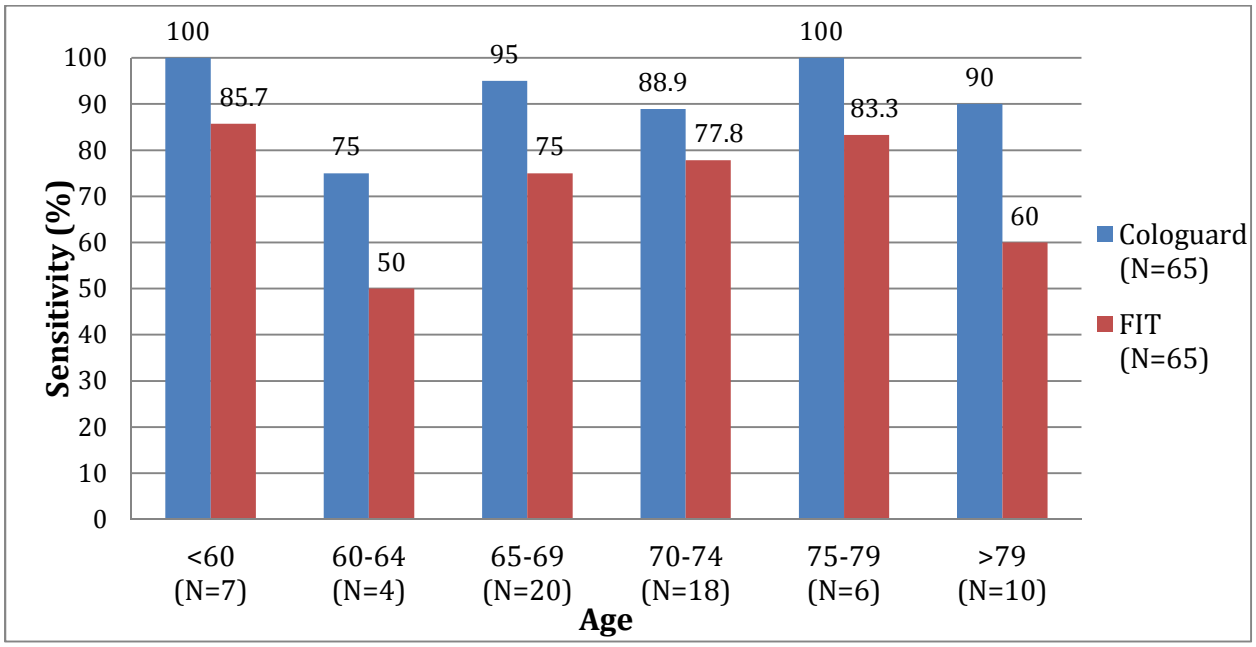
Category distributions by age for the subsets of the AA cases are presented in **Figure 11** below. As shown in the figure, across all categories the highest proportion of AA cases occurred in subjects 65-69 years old. For all types of AA, the majority of cases (approximately 60%) occurred in subjects 65-74 years old.

Figure 11: Category Distributions by Age, AA Cases – Primary Effectiveness Subjects



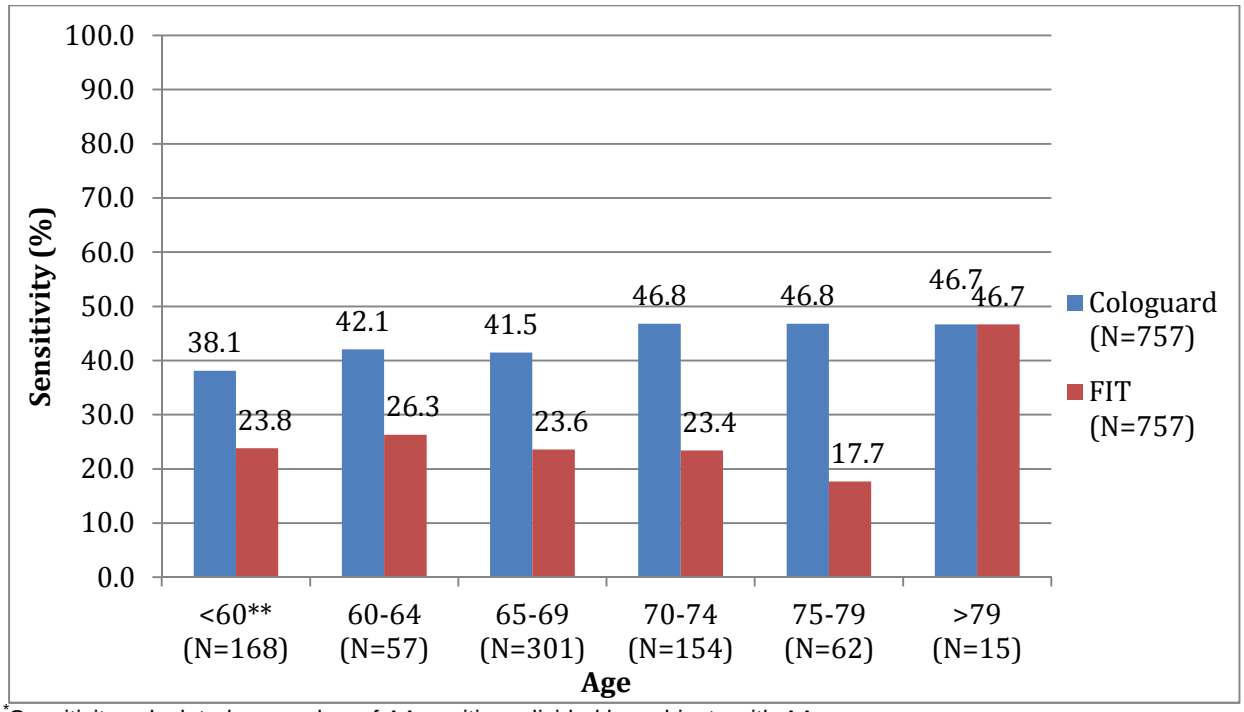
Cologuard sensitivity for CRC was consistently high across all age groups, as shown in **Figure 12** below. Sensitivity for patients 65 years of age and older ranged from 88.9% to 100.0%. Although sensitivity was 75% for subjects age 60-64, the number of CRC cases was particularly small in this age group (n = 4); only one CRC case was not detected by *Cologuard*. With respect to AA (**Figure 13**), sensitivity was similar for Cologuard across all age groups, with sensitivity as high as 46.8% for subjects 70-79 years old. In every age group, the sensitivity of FIT for CRC was numerically lower than that of *Cologuard*, although these comparisons were not statistically significant. Thus, even when divided by age group, *Cologuard* continued to demonstrate a higher sensitivity for CRC compared to FIT.

Figure 12: *Cologuard* and FIT CRC (Category 1) Sensitivity by Age
Secondary Effectiveness Subjects*



* Sensitivity calculated as number of CRC positives divided by subjects with CRC.

Figure 13: *Cologuard* and FIT AA (Category 2) Sensitivity by Age
Secondary Effectiveness Subjects*

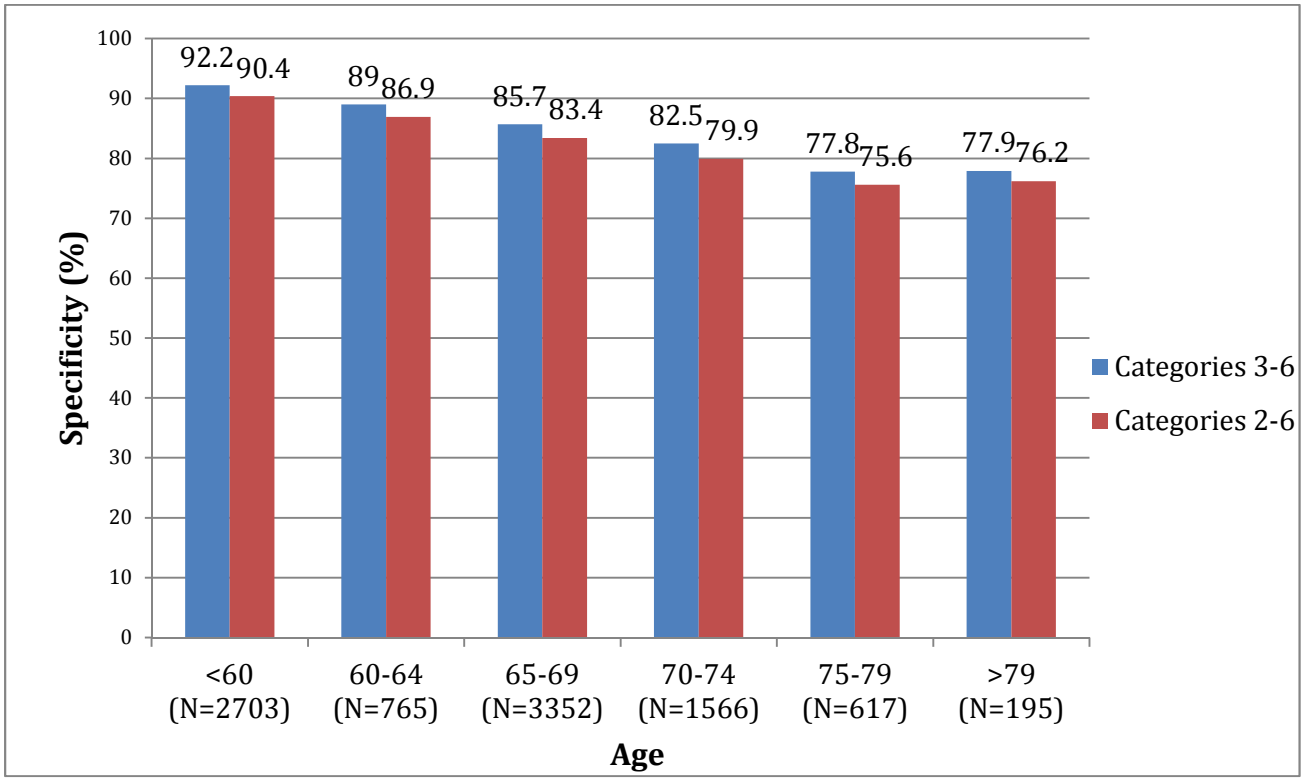


*Sensitivity calculated as number of AA positives divided by subjects with AA.
** N=168 in FIT group.

Given that the study population was age-enriched, Exact Sciences also analyzed the age-adjusted sensitivity of *Cologuard* for CRC and AA, using U.S. census data, to evaluate the effect of the age enrichment from the results. The age-adjusted sensitivity of *Cologuard* for CRC was 93.8%.

Cologuard specificity remained high across all age groups, both when AA cases were included in the analysis and excluded from the analysis, as shown in **Figure 14** below. In each analysis, specificity was highest for younger subjects and lower for older subjects; specificity was above 80% for most age groups, aside from subjects 75 years and older. Although there was a slight decrease in specificity among older subjects, the study was not powered to examine performance across multiple subgroups. Although specificity was slightly lower among older subjects, sensitivity was quite high. For example, *Cologuard* was positive for 9 out of 10 cancers in subjects over the age of 79. By comparison, FIT was positive for only 6 out of those 10 cancers. Thus, while specificity decreases somewhat among older subjects, *Cologuard* continued to detect the vast majority of cancer cases.

Figure 14: *Cologuard* Specificity by Age – Primary Effectiveness Subjects



Note: Specificity calculated as number of negatives among subjects without CRC or AA. Two 49-year-old and one 44 year old true negative subjects were included in the analysis population, although they would not be included in the intended user population.

The age-adjusted specificity of *Cologuard*, using U.S. census data, was 87.3%.

In addition, at FDA's request, Exact Sciences conducted an analysis of all subjects 65 years old or older. There were a total of 6,316 subjects 65 and older included in the Deep-C study, including 54 CRC cases and 522 Advanced Adenoma (AA) cases. As shown in Table 19 below, *Cologuard* sensitivity in this age group was comparable to that of the study population as a whole. *Cologuard* sensitivity for CRC was 92.6% for this age group, compared with 92.3% sensitivity for CRC for all DeeP-C subjects. Sensitivity of *Cologuard* for AA was also similar for subjects in this age group as

for the study population as a whole. *Cologuard* sensitivity for AA was 44.6% among subjects 65 and older, compared with 42.4% for the DeeP-C population overall.

Similarly, with respect to specificity, results were also comparable between the subject cohort 65 and older and the complete analysis population. As shown in **Table 20** below, specificity among the subjects 65 years old or older was 83.8% for categories 3-6. By comparison, specificity for categories 3-6 was 86.6% in the overall analysis population. For the alternative calculation examining categories 2-6, specificity was 81.5%, compared to 84.4% for the overall analysis population. Thus, specificity was a few points lower among subjects 65 and older compared to the overall population.

Table 19: *Cologuard* Sensitivity for Subjects ≥ 65 Years of Age

	Cologuard		
Findings on Colonoscopy	**N**	**N Positive**	**% Positive**
Category 1: Colorectal Cancer	54	50	92.6%
Category 2: Advanced Adenoma	522	233	44.6%

Table 20: *Cologuard* Specificity for Subjects ≥ 65 Years of Age

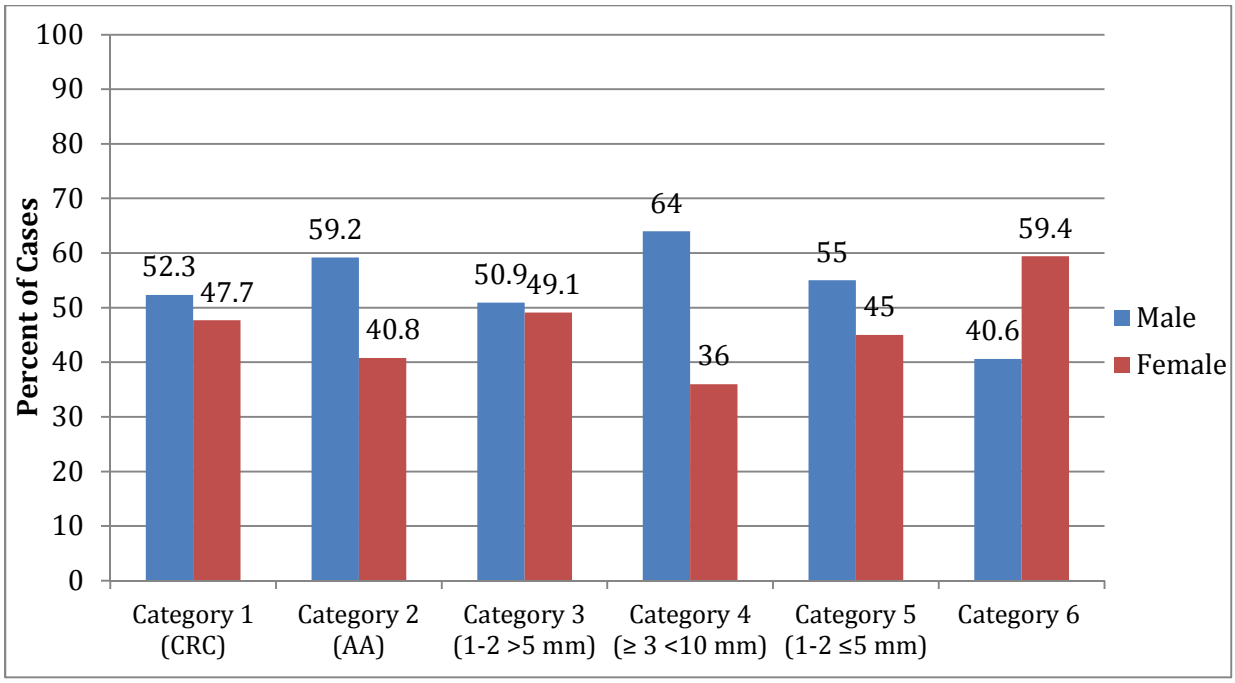
	Valid *Cologuard* Negative Result
Case Category, n/N (%)	
3: 1-2 Adenomas 5-<10 mm	383/495 (77.4%)
4: ≥3 Adenomas <10 mm, Non-advanced	249/329 (75.7%)
5: 1-2 Adenomas ≤5 mm, Non-advanced	961/1152 (83.4%)
6 (6.1 or 6.2) No colorectal neoplasia	3211/3754 (85.5%)
Specificity Based on Categories 3-6: Primary	4804/5730 (83.8%)
Specificity Based on Categories 2-6: Supportive	5093/6252 (81.5%)

7.8.2.2 Results by Gender

Cologuard and FIT data presented by gender subgroups is shown below. First, category distributions by gender for the Primary Effectiveness Population are presented in **Figure 15** below. As shown in the table, the gender distribution of CRC cases was similar (52.3% male compared with 47.7% female). For AA cases, a larger percentage of cases were male (450/760, 59.2%) compared with female (310/760, 40.8%). These gender distributions are consistent with the literature, as males have higher age-adjusted rates of adenoma than females.[71]

[71] Roy HK, Bianchi LK. (2009). Differences in Colon Adenomas in Men and Women: Potential Clinical Implications. *JAMA* 302(15):1696-1697. *See also* Villavicencio RT, Rex DK. (2005). Colonic adenomas: prevalence and incidence rates, growth rates, and miss rates at colonoscopy. *Semin Gastrointest Dis* 11:185–93 (noting adenoma prevalence was associated with male gender); Chen SC, Rex DK. (2007) Endoscopist Can Be More Powerful than Age and Male

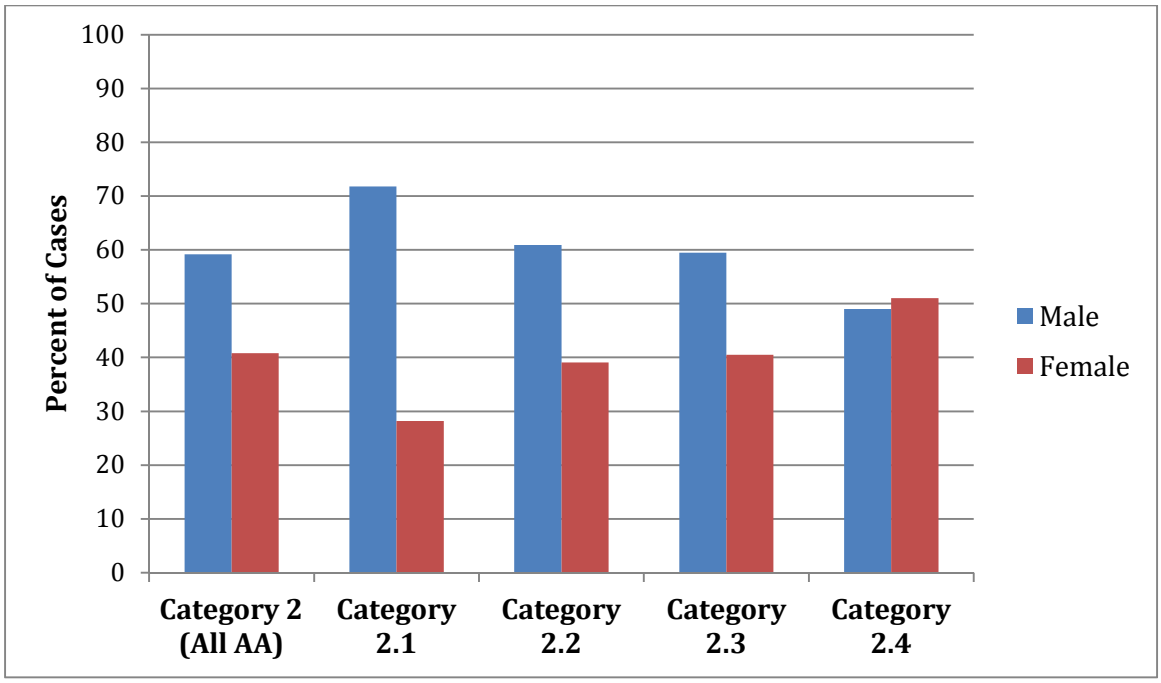
Figure 15: Category Distributions by Gender – Primary Effectiveness Subjects



Category distributions by gender for the subsets of the AA cases are also presented in **Figure 16** below. As shown in the table, aside from serrated lesions (Category 2.4) all other types of AAs were more common in men than women. Specifically, 71.8% (28/39) of Adenoma with carcinoma *in situ*/high grade dysplasia cases occurred in males. Similarly, approximately 60% of Adenoma with villous growth pattern cases ≥ 25% (156/256, 60.9%) and AAs ≥ 10 mm (217/365, 59.5%) occurred in male subjects.

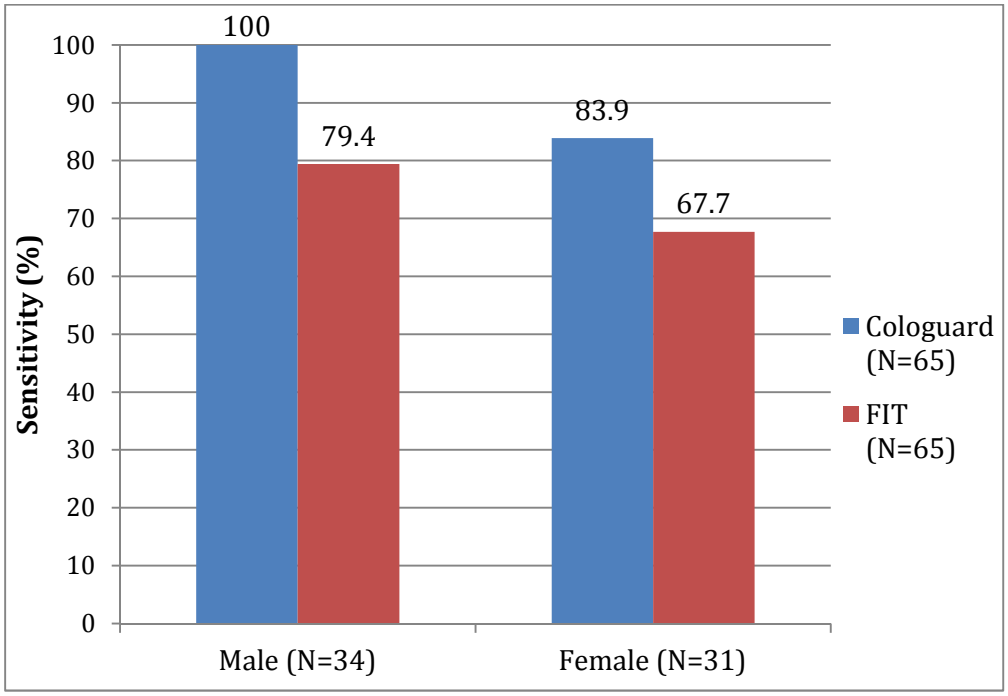
Gender in Predicting Adenoma Detection at Colonoscopy. *Amer J of Gastroenter* 102, 856–861 (noting that men had more adenomas than women).

Figure 16: Category Distributions by Gender, AA Cases – Primary Effectiveness Subjects



Sensitivity of *Cologuard* was higher for males than for females, both for CRC and AA. As shown in **Figure 17** below, *Cologuard* sensitivity for CRC was 100.0% for males, compared with 83.9% for females. A similar trend was observed for FIT where sensitivity for CRC was 79.4% for males compared to 67.7% for females.

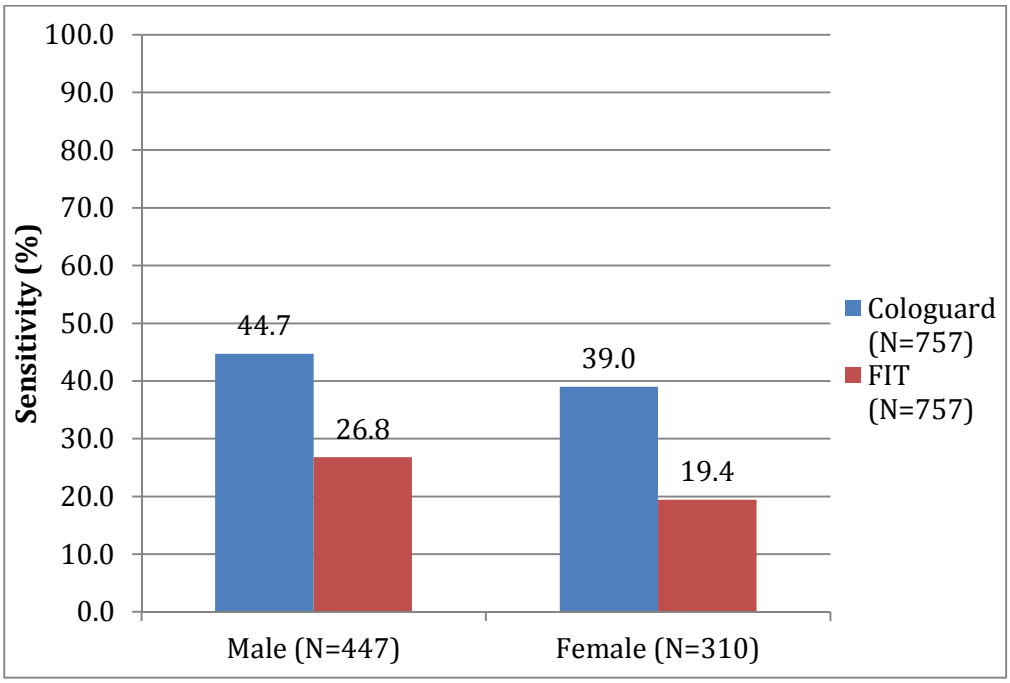
Figure 17: *Cologuard* and FIT CRC (Category 1) Sensitivity by Gender Secondary Effectiveness Population*



***Sensitivity calculated as number of CRC positives divided by subjects with CRC

Cologuard sensitivity for AA (**Figure 18**) was 44.7% for males, compared with 39.0% for females. Similarly, FIT sensitivity for AA was also higher among males (26.8%) compared to females (19.4%). Importantly, *Cologuard* sensitivity was approximately 15-20% higher than that of FIT, for both CRC and AA, across both genders.

Figure 18: *Cologuard* and FIT AA (Category 2) Sensitivity by Gender Secondary Effectiveness Population*

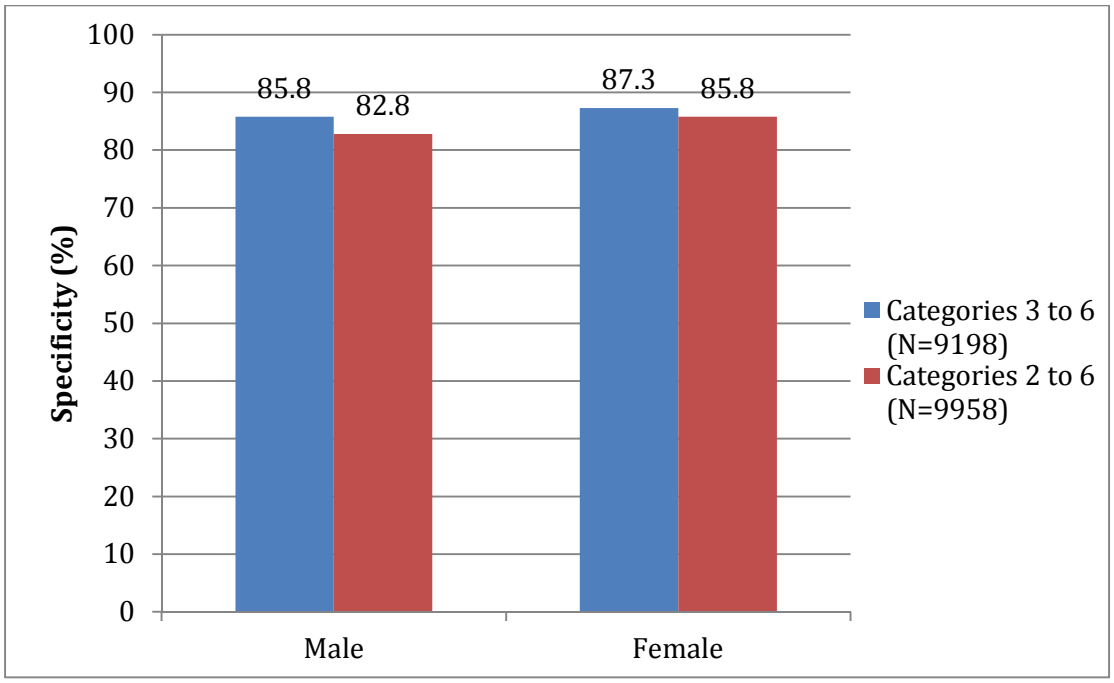


***Sensitivity calculated as number of AA positives divided by subjects with AA

It should be noted that only 5 CRC cases produced a negative *Cologuard* result. Although these cases happened to occur among female subjects, the overall number of cancer cases was relatively small. Given the number of different subgroups examined in the analysis of study data, it is expected that some subgroups would show decreased or increased sensitivity/specificity by chance, without a causative relationship. Notably, sensitivity was also decreased for FIT among women compared to men. Thus, it is possible that the lower sensitivity rates observed among women for both *Cologuard* and FIT were due to some other factor common to the women with cancer in whom *Cologuard* was negative. Exact Sciences reviewed the subjects' age, race, cancer stage, lesion size and location, but there were no notable trends.

Meanwhile, specificity of *Cologuard* was very similar for females as compared with males. As shown in **Figure 19** below, when AA cases were excluded from the analysis (Categories 3-6), specificity was 87.3% (4,398/5,037) for females, compared with 85.8% (3,569/4,161) for male subjects. When AA cases were included in the analysis (Categories 2-6), specificity was 85.8% (4,587/5,347) for female subjects, compared with 82.8% (3,818/4,611) for males.

Figure 19: *Cologuard* Specificity by Gender – Primary Effectiveness Subjects *



* Specificity calculated as number of negatives among subjects without CRC or AA.

7.8.2.3 Results by Race and Ethnicity

Results for *Cologuard* and FIT were also assessed by race and ethnicity. Categorization of subjects by race, ethnicity, and smoking history is shown in **Table 21** below, for the Primary Effectiveness Population. As shown in the table, the distribution of cases generally mirrors the overall study population. Specifically, the majority of CRC cases were Caucasian/White (55/65, 84.6%), as were the majority of subjects enrolled in the study. The next most common racial group represented in CRC cases was African American (8/65, 12.3% of CRC cases). A minority of CRC cases were Hispanic or Latino (9/65, 13.8%), which also mirrored the study population. With respect to AA, the racial and ethnic distribution of cases was similar to the CRC cases and the study population as a whole. The majority of AA cases were White/Caucasian (641/760, 84.5%) and were not Hispanic or Latino (700/760, 92.2%). As with CRC cases and the overall study population, the next most represented racial group among AA cases was African American (85/760, 11.2%). The racial and ethnic distribution of subjects in Categories 3-6 was similar to that of CRC and AA cases. Subjects categorized as "other" includes subjects of mixed racial backgrounds or subjects who reported their ethnicity in the "other" category.

In comparison to the overall study population, there were slightly more Hispanic or Latino subjects represented in the CRC cases (9/65, 13.8%) than in the primary effectiveness population as a whole (999/10,023, 9.9%). Similarly, the representation of Black/African-Americans in the CRC cases (8/65, 12.3%) was slightly higher than the analysis population as a whole (1,071/10,023, 10.7%). The higher representation of Black/African-American subjects in the CRC cases is consistent with the most recent data available from the Centers for Disease Control and Prevention, which show

that CRC incidence is highest among Black/African-Americans, followed by White/Caucasians, and then Hispanics.[72]

Category distributions by race and ethnicity for the subsets of the AA cases are presented in **Table 21** below. As shown in the table, with respect to race, the distribution of cases was similar across racial and ethnic groups for most types of AA. A higher proportion of serrated lesion cases were White (94/100, 94%) than other types of AA. As for ethnicity, the proportion of Adenoma with carcinoma *in situ*/high grade dysplasia cases that were Hispanic or Latino subjects was higher (6/39, 15.4%) than for any other types of AA.

[72] Centers for Disease Control and Prevention, Colorectal Cancer Rates by Race and Ethnicity, 2010, *available at*: http://www.cdc.gov/cancer/colorectal/statistics/race.htm.

Table 21: Category Distribution by Race and Ethnicity – Primary Effectiveness Subjects

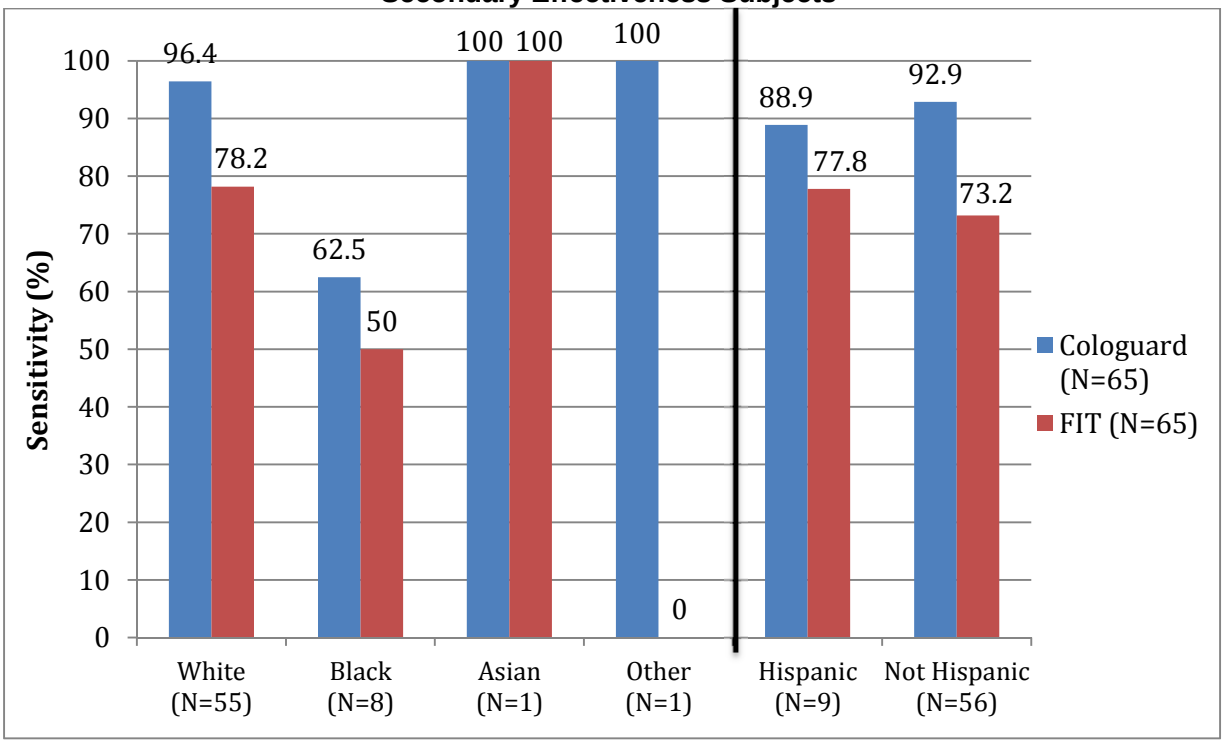
Subgroup	Category 1 (CRC) (N=65)	Category 2 (AA) (N=760)	Category 3 (1-2 >5 mm) (N=749)	Category 4 (≥3 <10 mm) (N=419)	Category 5 (1-2 ≤5 mm) (N=1735)	Category 6 (6.1 or 6.2) (N=6295)
Race, n (%)						
White/Caucasian	55 (84.6)	641 (84.5)	640 (85.6)	351 (83.8)	1468 (84.7)	5267 (83.7)
Black or African American	8 (12.3)	85 (11.2)	77 (10.3)	39 (9.3)	185 (10.7)	677 (10.8)
Asian	1 (1.5)	13 (1.7)	20 (2.7)	11 (2.6)	45 (2.6)	169 (2.7)
American Indian or Alaska Native	0 (0.0)	4 (0.5)	2 (0.3)	4 (1.0)	4 (0.2)	22 (0.3)
Native Hawaiian or Other Pacific Islander	0 (0.0)	0 (0.0)	1 (0.1)	1 (0.2)	4 (0.2)	17 (0.3)
Other	1 (1.5)	16 (2.1)	8 (1.1)	13 (3.1)	28 (1.6)	140 (2.2)
Missing	0	1	1	0	1	3
Ethnicity, n (%)						
Hispanic or Latino	9 (13.8)	59 (7.8)	78 (10.4)	50 (11.9)	171 (9.9)	624 (9.9)
Not Hispanic or Latino	56 (86.2)	700 (92.2)	671 (89.6)	369 (88.1)	1563 (90.1)	5669 (90.1)
Missing	0	1	0	0	1	2

Table 22: Category Distributions by Race and Ethnicity, AA Cases – Primary Effectiveness Subjects

Subgroup	Category 2 (All AA) (N=760)	Category 2.1 (Adenoma with carcinoma *in situ*/HGD) (N=39)	Category 2.2 (Adenoma, villous growth pattern >/= 25%) (N=256)	Category 2.3 (Adenoma ≥10 mm) (N=365)	Category 2.4 (Serrated Lesion ≥10 mm) (N=100)
Race, n (%)					
White/Caucasian	641 (84.5)	32 (82.1)	208 (81.6)	307 (84.1)	94 (94.0)
Black or African American	85 (11.2)	5 (12.8)	34 (13.3)	43 (11.8)	3 (3.0)
Asian	13 (1.7)	1 (2.6)	7 (2.7)	4 (1.1)	1 (1.0)
American Indian or Alaska Native	4 (0.5)	1 (2.6)	2 (0.8)	1 (0.3)	0 (0.0)
Native Hawaiian or Other Pacific Islander	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Other	16 (2.1)	0 (0.0)	4 (1.6)	10 (2.7)	2 (2.0)
Missing	1	0	1	0	0
Ethnicity, n (%)					
Hispanic or Latino	59 (7.8)	6 (15.4)	18 (7.1)	30 (8.2)	5 (5.0)
Not Hispanic or Latino	700 (92.2)	33 (84.6)	237 (92.9)	335 (91.8)	95 (95.0)
Missing	1	0	1	0	0

With respect to race, as shown in **Figure 20**, *Cologuard* sensitivity for CRC was very high among White/Caucasians (53/55, 96.4%). Among Black or African-Americans the observed sensitivity was lower, but the number of CRC cases was small (5/8, 62.5%). A similar trend was observed for FIT where sensitivity was higher for White/Caucasians (43/55, 78.2%) than African American subjects (4/8, 50.0%). *Cologuard* sensitivity among Hispanic or Latinos was 88.9% (8/9), although again, sample size was small. FIT sensitivity for CRC among Hispanic or Latinos (7/9, 77.8%) was similar to that of non-Hispanic or Latinos (73.2%). Notably, *Cologuard* sensitivity was higher than FIT for CRC across all racial and ethnic subgroups with the exception of Asian subjects, where both tests were positive for the single Asian CRC subject.

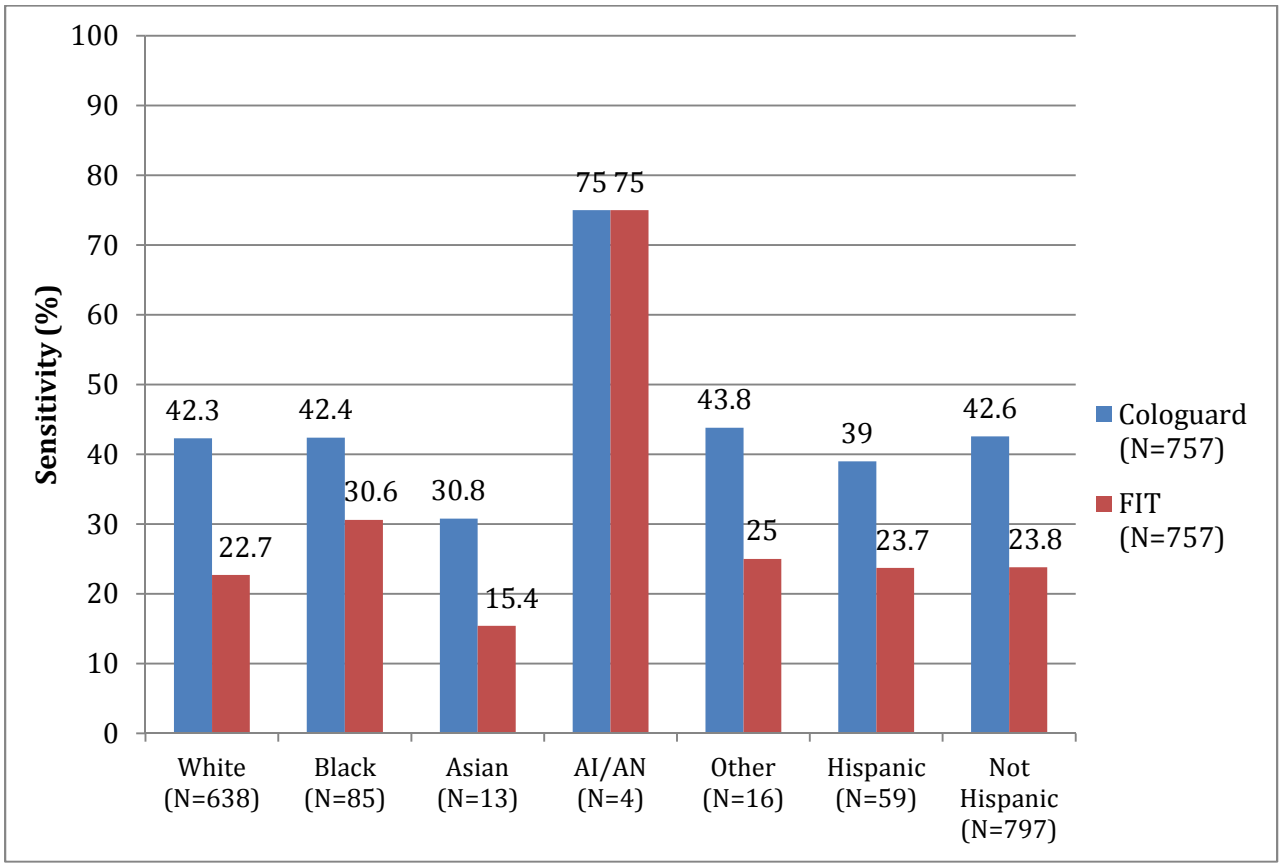
Figure 20: *Cologuard* and FIT CRC (Category 1) Sensitivity by Race & Ethnicity Secondary Effectiveness Subjects*



*Sensitivity calculated as number of CRC positives divided by subjects with CRC. There were no CRC cases among American Indian/Alaska Native or Native Hawaiian/Other Pacific Islander subjects.

Cologuard sensitivity for AA by race and ethnicity are shown in **Figure 21** below. *Cologuard* sensitivity for AA was similar for White/Caucasians (270/638, 42.3%) and Black/African-Americans (36/85, 42.4%). Meanwhile FIT sensitivity for Black/African American subjects (30.6%) as higher than for White/Caucasians (22.7%). *Cologuard* sensitivity was similar among Hispanic/Latino subjects (39.0%) compared to non-Hispanics (42.6%). *Cologuard* sensitivity for AA was lower among Asians (4/13, 30.8%) and high for American Indian or Alaskan Natives (3/4, 75.0%), compared with other groups. FIT sensitivity for CRC among Hispanic or Latinos was similar to that of non-Hispanic or Latinos (23.7% compared with 23.8%, respectively). Similar to results for CRC, *Cologuard* sensitivity for AA was higher than FIT across all racial and ethnic subgroups, except for American Indian/Alaska Natives where both tests were positive for 3 of the 4 AA cases.

Figure 21: *Cologuard* and FIT AA (Category 2) Sensitivity by Race & Ethnicity Secondary Effectiveness Subjects*

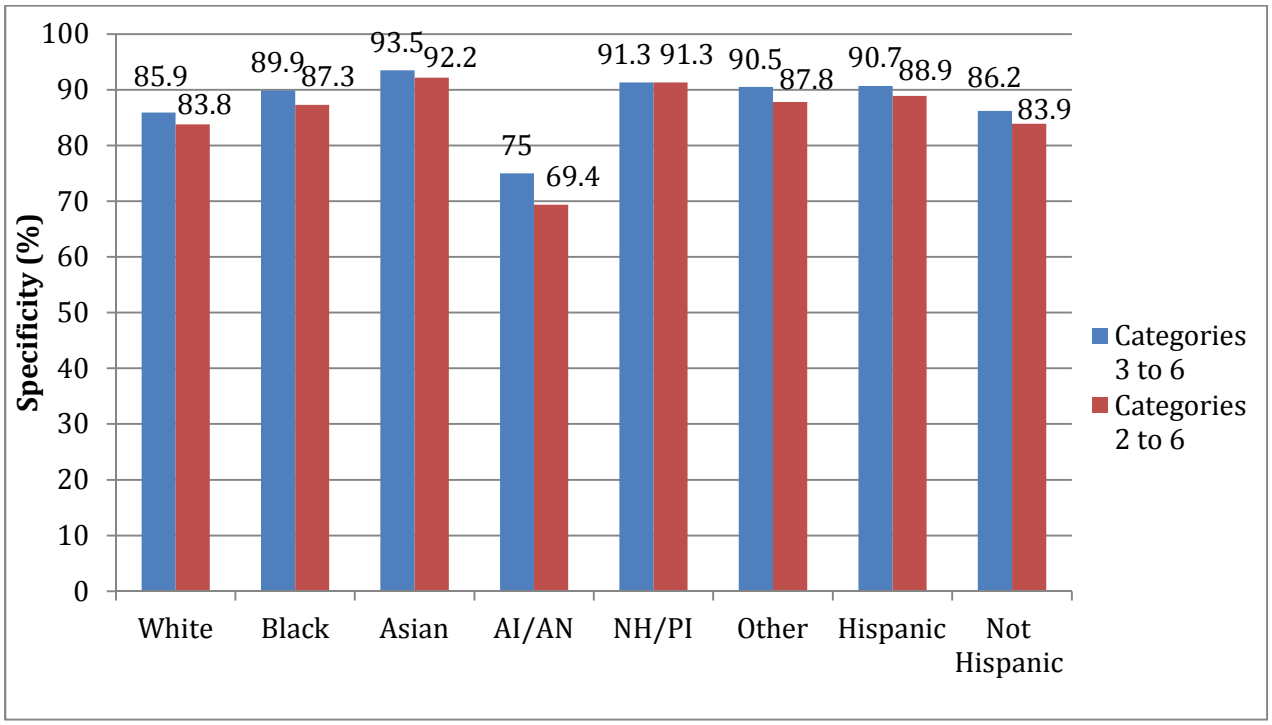


*Sensitivity calculated as number of AA positives divided by subjects with AA. There were no AA cases among Native Hawaiian/Other Pacific Islander subjects.

Cologuard specificity was high across all racial and ethnic groups, with rates > 85% for most groups. Specificity rates were highest for Asian and Native Hawaiian/Pacific Islanders and lowest for American Indian/Alaska Natives, as shown in **Figure 22** below.[73] Results were very similar in the alternative analysis including AA cases as true negatives.

[73] Subjects in the "other" category included subjects of mixed race or who reported their ethnicity rather than race.

Figure 22: *Cologuard* Specificity by Race/Ethnicity
Primary Effectiveness Subjects*



* Specificity calculated as number of negatives among subjects without CRC or AA.

7.8.2.4 Results by Lesion Size and Cancer Stage

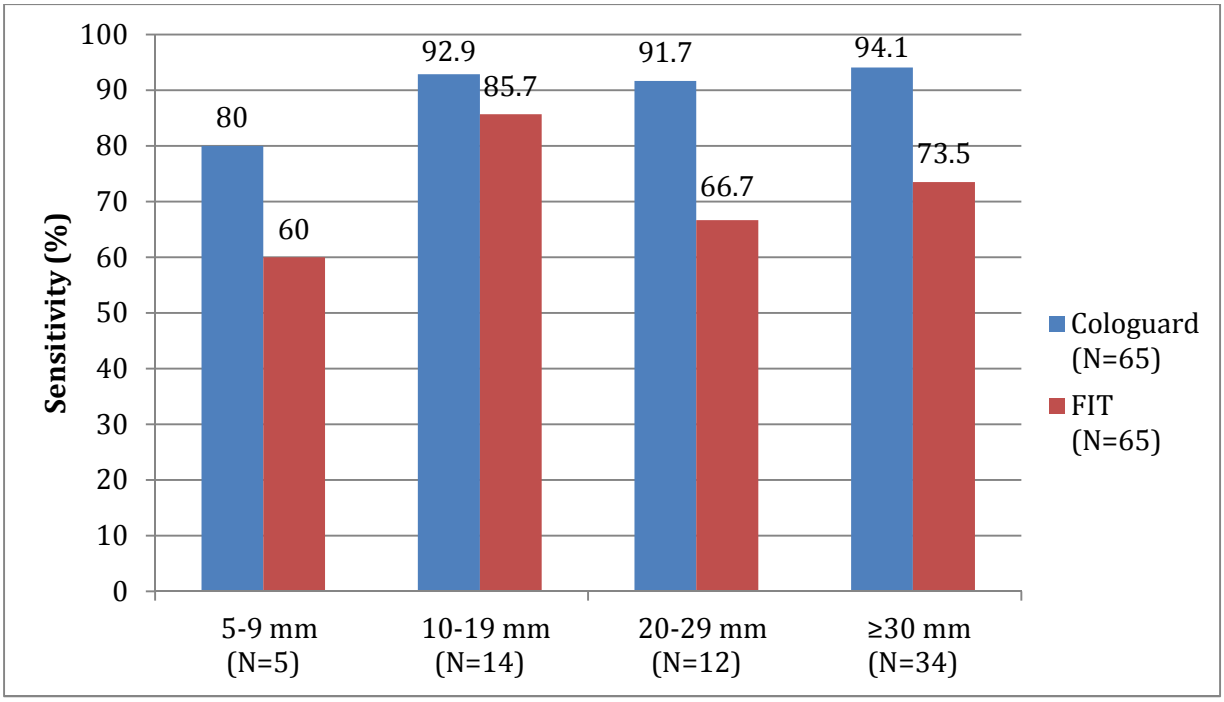
Study results were also analyzed by lesion size, as well as cancer stage. For CRC cases, slight majority were ≥ 30 mm in size (34/65, 52.3%). For AA subjects, 75.9% (577/760) of AAs were 10-19 mm in size. As could be expected by the definitions for each category, subjects in Categories 3-6 had either small hyperplastic or adenomatous lesions (< 10 mm in size), or no lesions. As for staging, 44.6% (29/65) of CRC subjects were Stage I cases, 32.3% (21/65) were Stage II cases, 15.4% (10/65) were Stage III cases, and 6.2% (4/65) were Stage IV cases. One case remained unstaged (Stage X) on completion of the study.

Subset category distributions of the AA cases by size showed that, across all types of AA, the largest proportion of cases involved lesions 10-19 mm in size. Notably, median lesion size was larger for subjects with Adenoma with carcinoma *in situ*/high grade dysplasia (Category 2.1). The majority of Adenoma with carcinoma *in situ*/high grade dysplasia cases occurred in lesions >1 cm (29/39, 74.4%) rather than lesions <1 cm (10/39, 25.6%). Almost all AA ≥10 mm cases (Category 2.3) and serrated lesion cases (Category 2.4) were 10-19 mm lesions.

As shown in **Figure 23** below, sensitivity of *Cologuard* increased with tumor size. This is biologically rational and consistent, as tumor detection by the stool-based DNA test is influenced by the amount cells shed (or exfoliated) from the tumor surface and larger lesions are associated with more shedding.

Cologuard CRC sensitivity was > 90% for most lesion sizes. Sensitivity was highest for subjects with CRCs ≥ 30 mm (32/34, 94.1%) and lowest for subjects with CRCs 5-9 mm in size (4/5, 80.0%). Meanwhile, for FIT the highest CRC sensitivity was among subjects with CRC 10-19 mm (85.7%).

Figure 23: *Cologuard* and FIT CRC (Category 1) Sensitivity by Lesion Size Secondary Effectiveness Subjects*

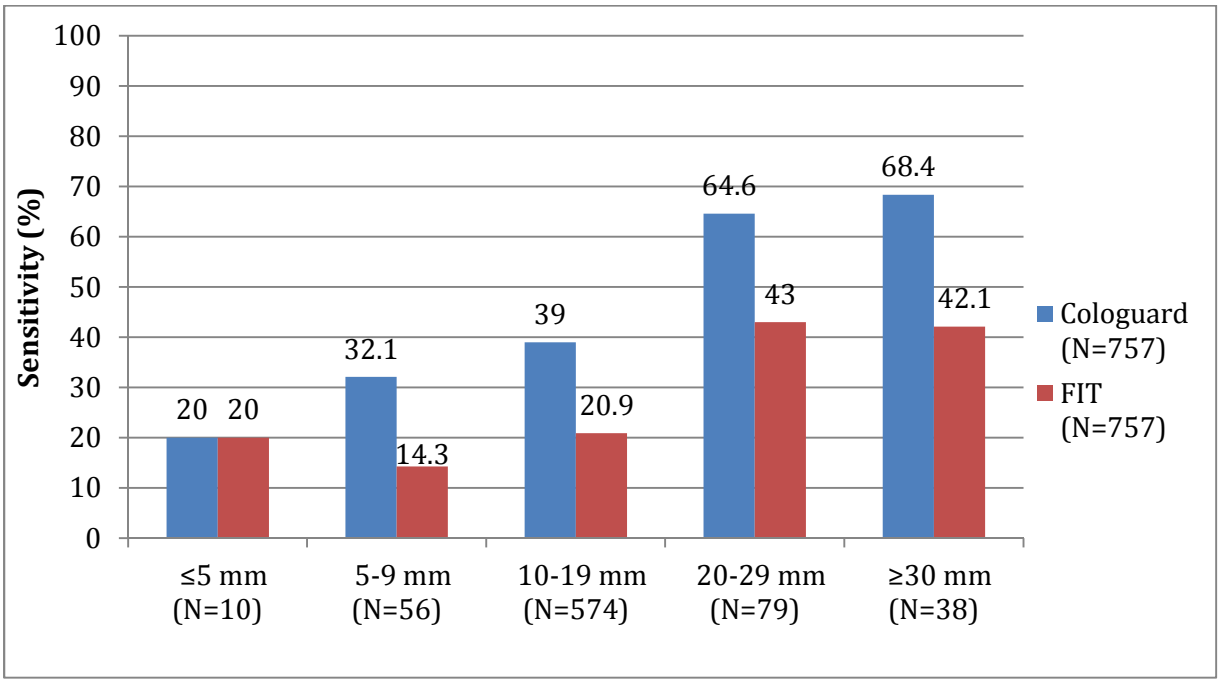


* Sensitivity calculated as number of CRC positives divided by subjects with CRC.

As shown in **Figure 24** below, sensitivity of *Cologuard* for AA was also higher among subjects with AAs of larger sizes, which are more likely to progress to cancer.[74] Sensitivity was 64.1% (51/79) for subjects with AAs 20-29 mm and 68.4% (26/38) for subjects with AAs ≥ 30 mm in size. Thus, *Cologuard* sensitivity is highest in those larger AAs, which are at the greatest risk of progression to CRC. By comparison, FIT sensitivity for AA was slightly higher among subjects with lesions 20-29 mm compared to other sizes.

[74] Muto T, Bussey JR, Morson, BC (1975). The Evolution of Cancer of the Colon and Rectum. *Cancer* (36) 2251-227.

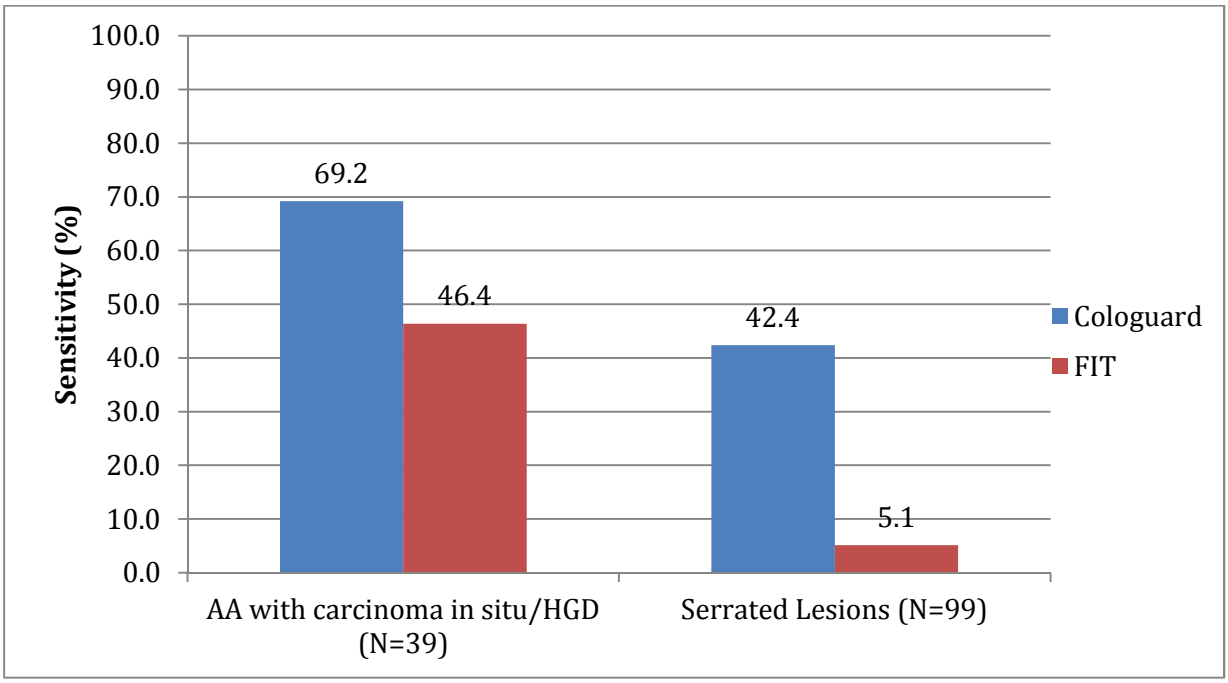
Figure 24: *Cologuard* and FIT AA (Category 2) Sensitivity by Lesion Size Secondary Effectiveness Subjects*



*Sensitivity calculated as number of AA positives divided by subjects with AA.

As noted previously in **Section 7.5** above, *Cologuard* offered a sensitivity advantage over FIT for Adenoma with carcinoma *in situ*/high grade dysplasia and serrated lesions, two significant CRC pathways. As shown in the figure below, sensitivity of *Cologuard* for Adenoma with carcinoma *in situ*/high grade dysplasia was 69.2%, compared with 46.4% for FIT. *Cologuard* sensitivity for serrated lesions was 42.4%, compared with 5.1% for FIT.

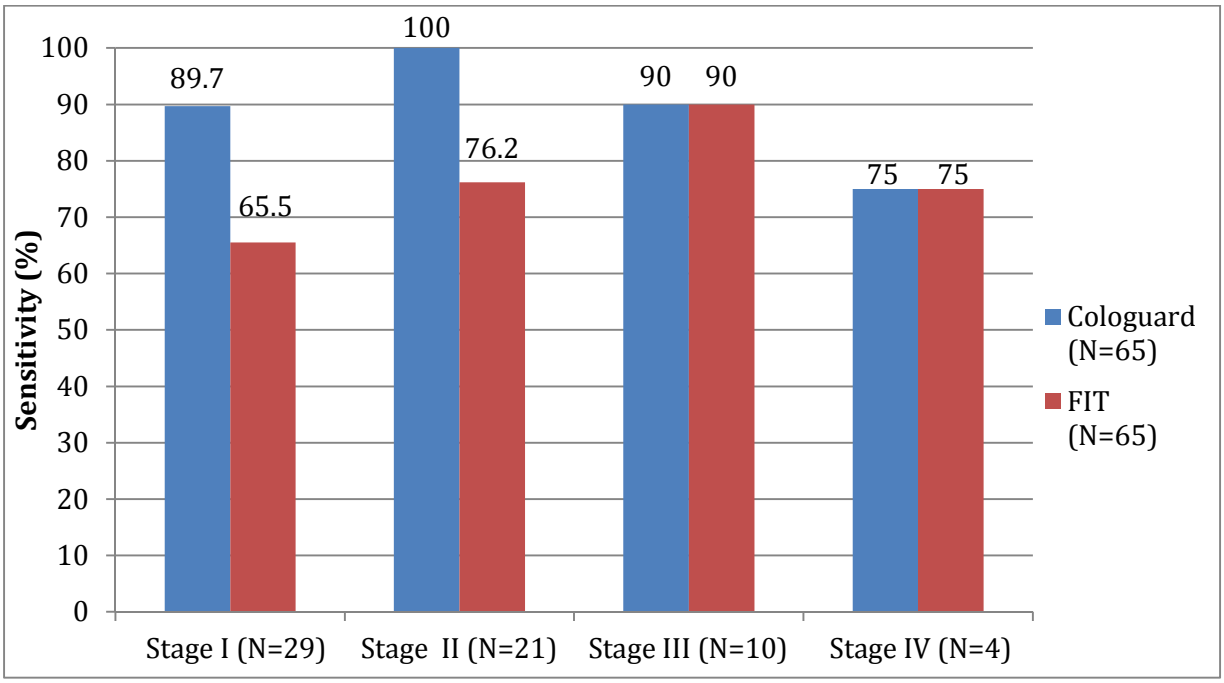
Figure 25: *Cologuard* and FIT AA Sensitivity by Lesion Type (Categories 2.1 and 2.4) Secondary Effectiveness Subjects*



*Sensitivity calculated as number of AA positives divided by subjects with AA.

Importantly, *Cologuard* outperformed FIT not only overall, but particularly in rescectable, early stage cancers. As shown in **Figure 26**, *Cologuard* sensitivity by cancer stage was highest for subjects with Stage II cancers (21/21, 100.0%) and Stage III cancers (9/10, 90%). Meanwhile, sensitivity of FIT was generally lower than *Cologuard*, aside from subjects in Stage IV, for whom sensitivity of both tests was 75.0%. Unlike *Cologuard*, FIT sensitivity was lower for early stage CRC cases than in more advanced stage CRC. Thus, *Cologuard* identified more cancers earlier, at which point the likelihood of survival and the benefit of screening is greatest.

Figure 26: *Cologuard* and FIT CRC (Category 1) Sensitivity by Cancer Stage Secondary Effectiveness Subjects



Specificity of *Cologuard* with respect to subjects with small, non-advanced adenomas (Category 3-5) and Hyperplastic polyps (Category 6) was high. Importantly, subjects with < 1 cm lesions were considered "normal." When AA cases were excluded from the analysis, specificity of *Cologuard* was 86.2% (1,847/2,142), for subjects with lesions < 5 mm in size, and 79.7% (1,523/1,912) for subjects with lesions 5-9 mm in size. Specificity for subjects with no findings on colonoscopy (Category 6.2) was 89.9% (4,019/4,474) for *Cologuard*.

When AA cases were included in the analysis, specificity of *Cologuard* was 86.2% (1,855/2,152) for subjects with lesions < 5 mm in size and 79.3% (1,561/1,968) for subjects with lesions 5-9 mm in size.

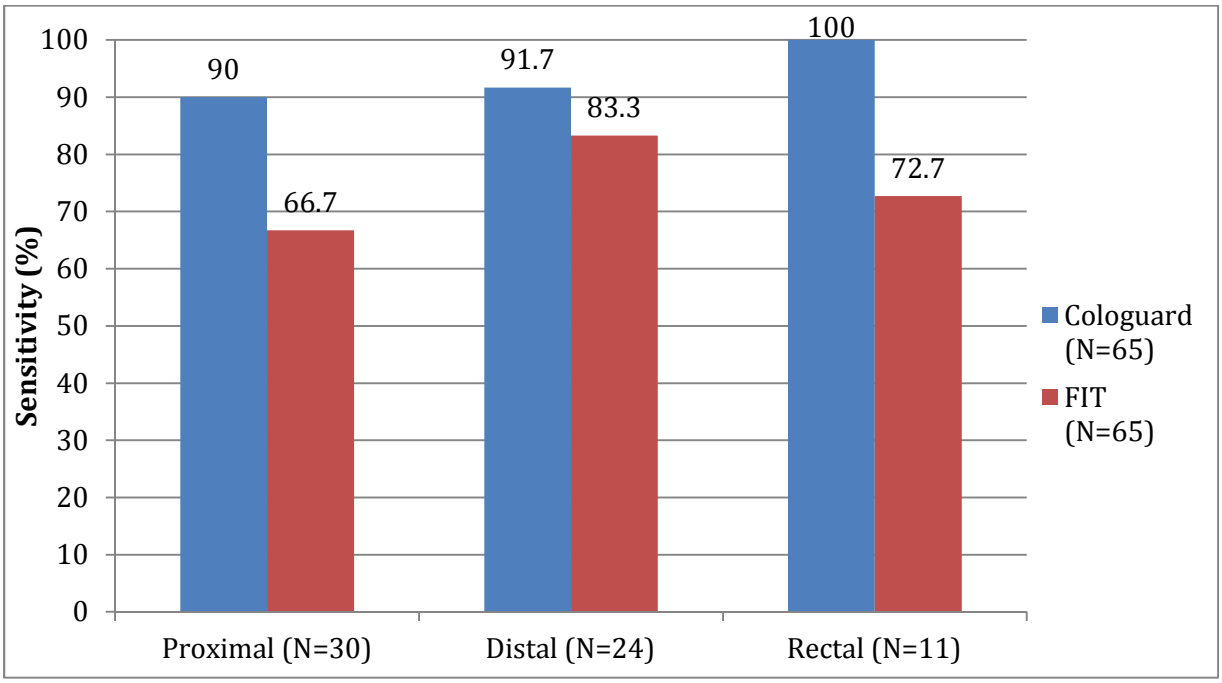
7.8.2.5 Results by Lesion Location

Cologuard and FIT results were also assessed by lesion location. It should be noted that the study captured lesion location as proximal, distal or rectal. While it is recognized that results reported in the literature are often reported only as proximal or distal (with recal included in distal), results reported here are consistent with the categories provided in the study protocol. More than half (431/757, 56.9%) of AA subjects had a proximal AA. For subjects in Categories 3-6, the majority of the lesions also were proximal in location. Adenomas with carcinoma *in situ*/high grade dysplasia (Category 2.1) cases were evenly distributed among proximal, distal, and rectal locations. Meanwhile, for all other categories, the majority of AAs were located proximally.

The results of *Cologuard* and FIT sensitivity by lesion location are shown in **Figure 27** and **Figure 28**. Sensitivity of *Cologuard* for CRC was 90% or greater, regardless of CRC location. Meanwhile, FIT sensitivity for CRC was lower than *Cologuard*, regardless of lesion location.

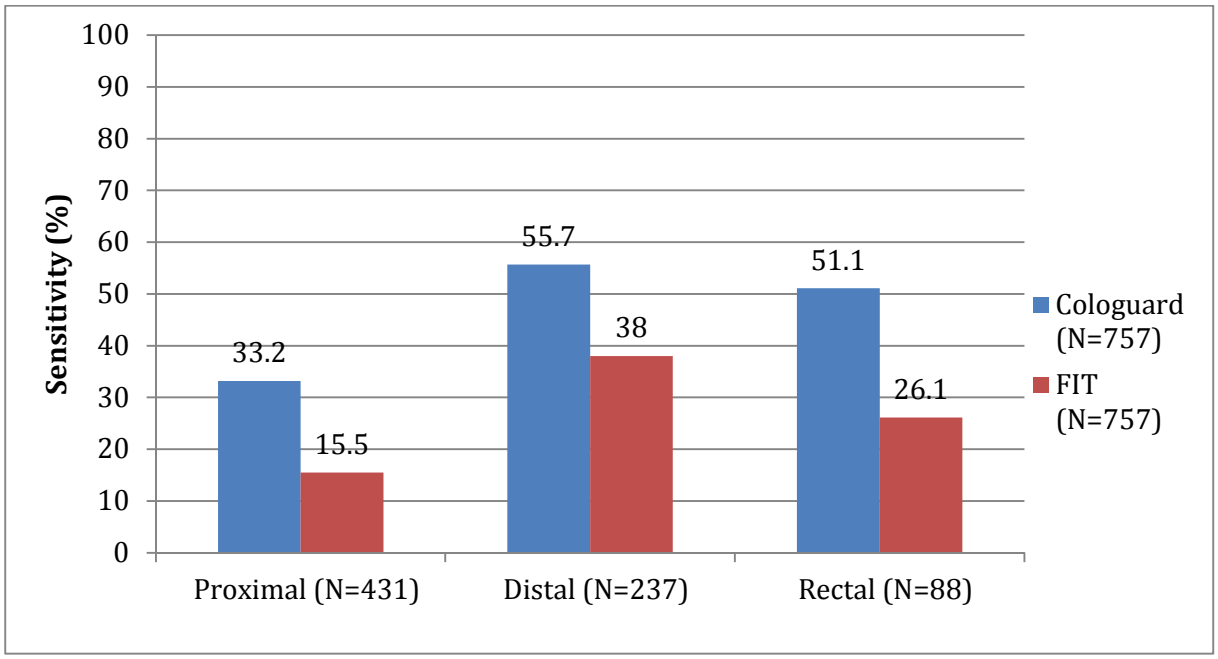
Sensitivity of *Cologuard* for AA was greatest among subjects with distal AAs (132/237, 55.7%). For FIT, AA sensitivity rates were lower than those of *Cologuard* for subjects with proximal AAs (67/431, 15.5%), distal AAs (90/237, 38.0%), and rectal AAs (23/88, 26.1%).

Figure 27: *Cologuard* and FIT CRC (Category 1) Sensitivity by Lesion Location Secondary Effectiveness Subjects*



* Sensitivity calculated as number of CRC positives divided by subjects with CRC.

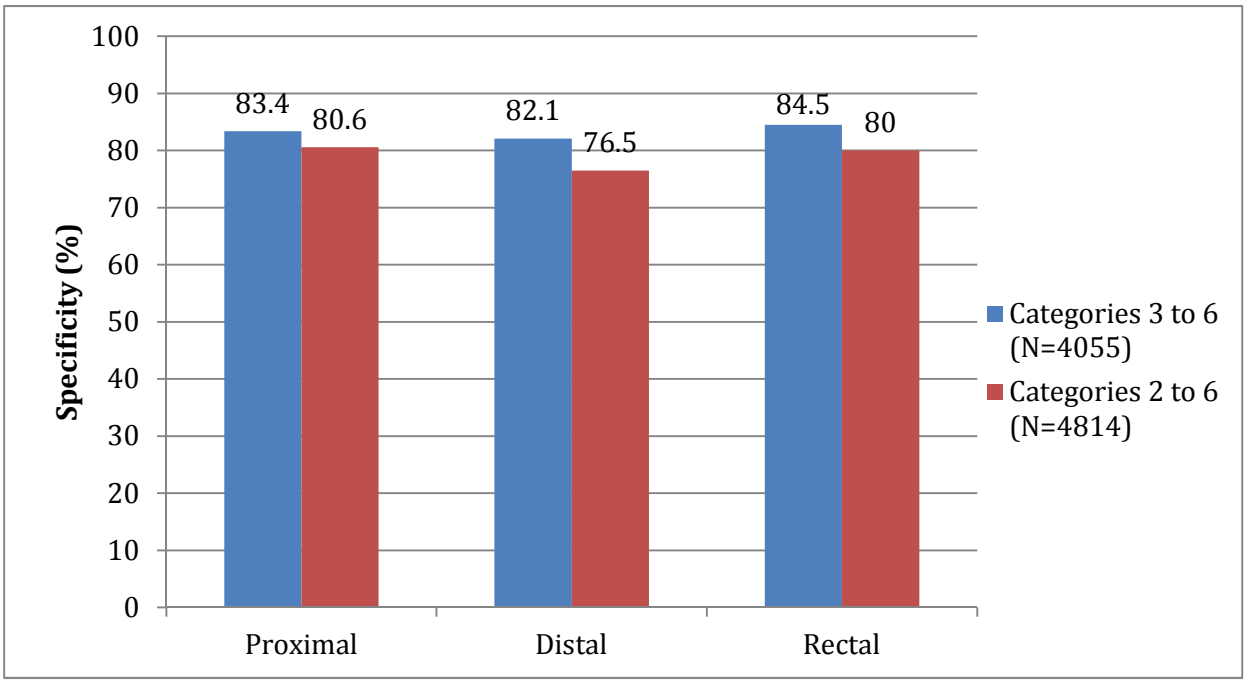
Figure 28: *Cologuard* and FIT AA (Category 2) Sensitivity by Lesion Location Secondary Effectiveness Subjects*



* Sensitivity calculated as number of AA positives divided by subjects with AA.

As shown in **Figure 29**, specificity of *Cologuard* was high, regardless of lesion location. Examining only subjects with a lesion[75] in Categories 3 through 5, specificity of *Cologuard* was 83.4% (1,723/2,066) for subjects with proximal lesions, 82.1% (1,131/1,377) for subjects with distal lesions, and 84.5% (517/612) for subjects with rectal lesions. Considering subjects with lesions in Categories 2 through 6, specificity was approximately 80% for subjects with proximal lesions and rectal lesions. However, specificity was slightly lower for subjects with distal lesions, (1,236/1,615) 76.5%.

Figure 29: *Cologuard* Specificity by Lesion Location (Specificity Subsets: Categories 3-6 and 2-6) – Primary Effectiveness Subjects



Note: Excludes subjects with no lesion
* Specificity calculated as number of negatives among subjects without CRC or AA.

7.8.2.6 Results by Clinic Type and Laboratory

Results were further analyzed by clinic type, both with respect to size and whether the site was a primary care or colonoscopy site. In addition, results were also analyzed according to the lab that processed the *Cologuard* test. The majority of CRC cases were from colonoscopy sites (58/65, 89.2%), as opposed to primary care sites (7/65, 10.8%). The majority of CRC cases were also enrolled at sites with ≥ 100 subjects (58/65, 89.2%), as opposed to smaller sites (7/65, 10.8%). With respect to laboratory, 18.5% of CRC case samples were processed at Lab 1, 64.6% at Lab 2, and 16.9% at Lab 3. This is consistent with the pre-planned distribution of samples to these labs. To ensure that all laboratories had the opportunity to process some positive CRC or AA cases, Exact Sciences intentionally distributed samples that were positive on colonoscopy proportionally to the labs, according to a pre-specified distribution plan.

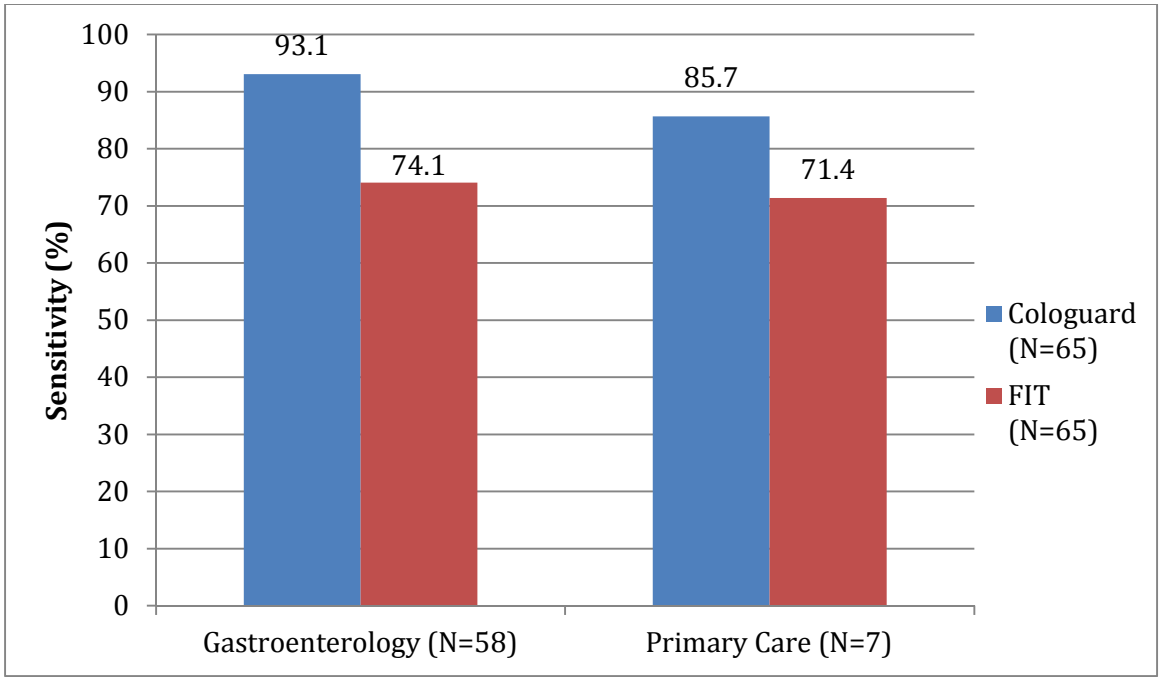
[75] Note that subjects without any lesions could not be categorized by location and, therefore, are excluded from this analysis.

The distribution of AA subjects was similar to that of CRC subjects. The majority of AA cases were from colonoscopy sites (672/760, 88.4%) and from sites with ≥ 100 subjects (659/760, 86.7%). As with CRC cases, more than half of AA cases were identified from samples processed at Lab 2 (456/760, 60.0%). AA cases were also distributed to the Labs according to the same pre-specified distribution plan used for CRC cases.

Category distributions by clinic type and size showed that the distribution of AA cases matches that of the whole primary effectiveness population. Specifically, the majority of AA cases, regardless of type, were identified at colonoscopy sites. The majority of AA cases, regardless of type, were identified at sites that enrolled ≥ 100 subjects. The proportion of cases identified at each laboratory was similar for AA cases as CRC cases.

Sensitivity results for *Cologuard* and FIT were similar for primary care and gastroenterology specialty sites. As shown in **Figure 30**, sensitivity for CRC was 85.7% (6/7) at primary care sites, compared with 93.1% (54/58) for gastroenterology specialty sites.

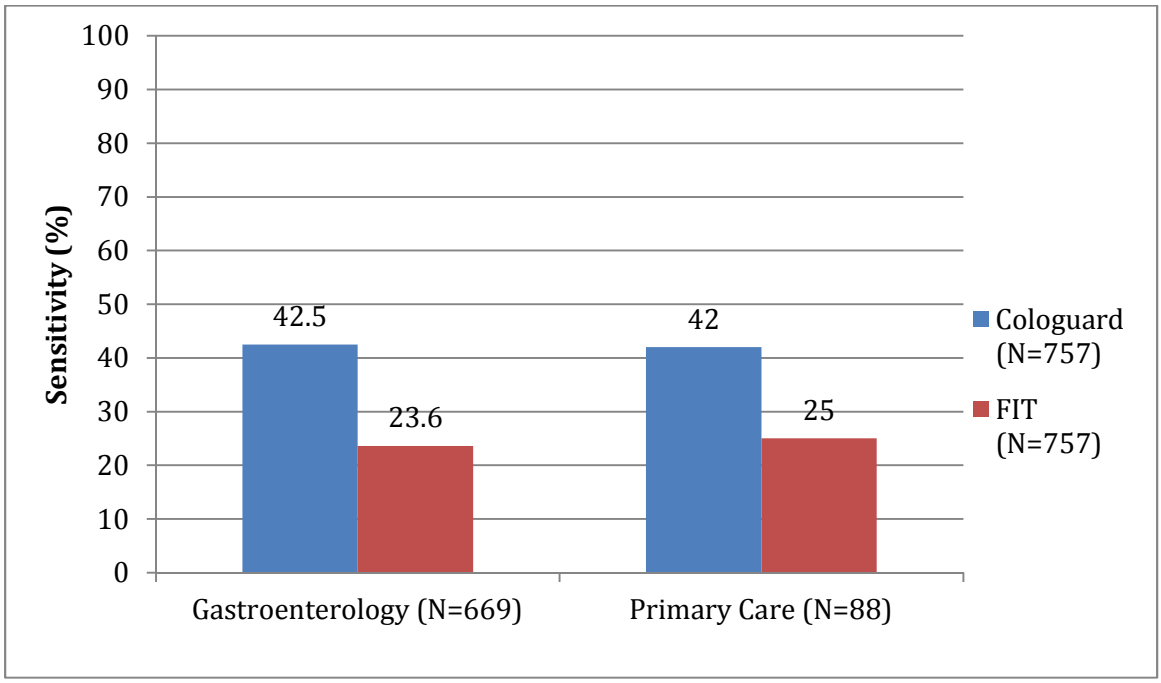
Figure 30: *Cologuard* and FIT CRC (Category 1) Sensitivity by Point of Referral Site Secondary Effectiveness Subjects*



* Sensitivity calculated as number of CRC positives divided by subjects with CRC.

As shown in **Figure 31**, sensitivity for AA was 42.0% (37/88) at primary care sites, compared with 42.4% (285/672) for gastroenterology specialty sites.

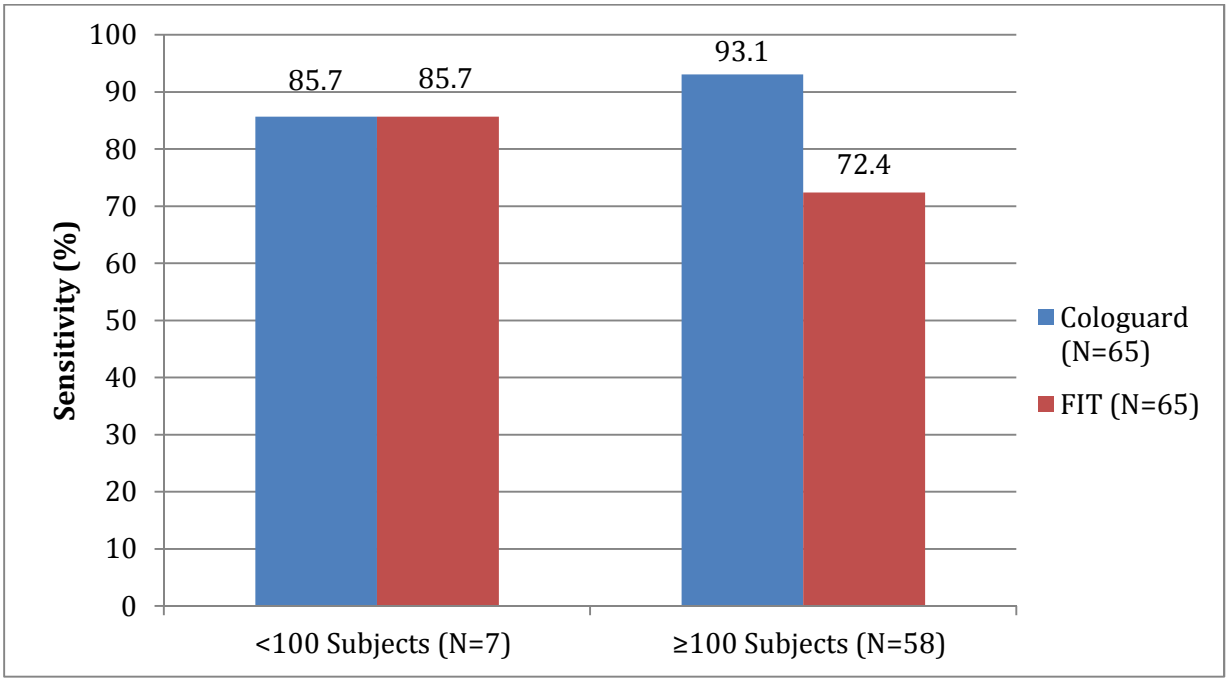
Figure 31: *Cologuard* and FIT AA (Category 2) Sensitivity by Point of Referral Site Secondary Effectiveness Subjects*



***** Sensitivity calculated as number of AA positives divided by subjects with AA.

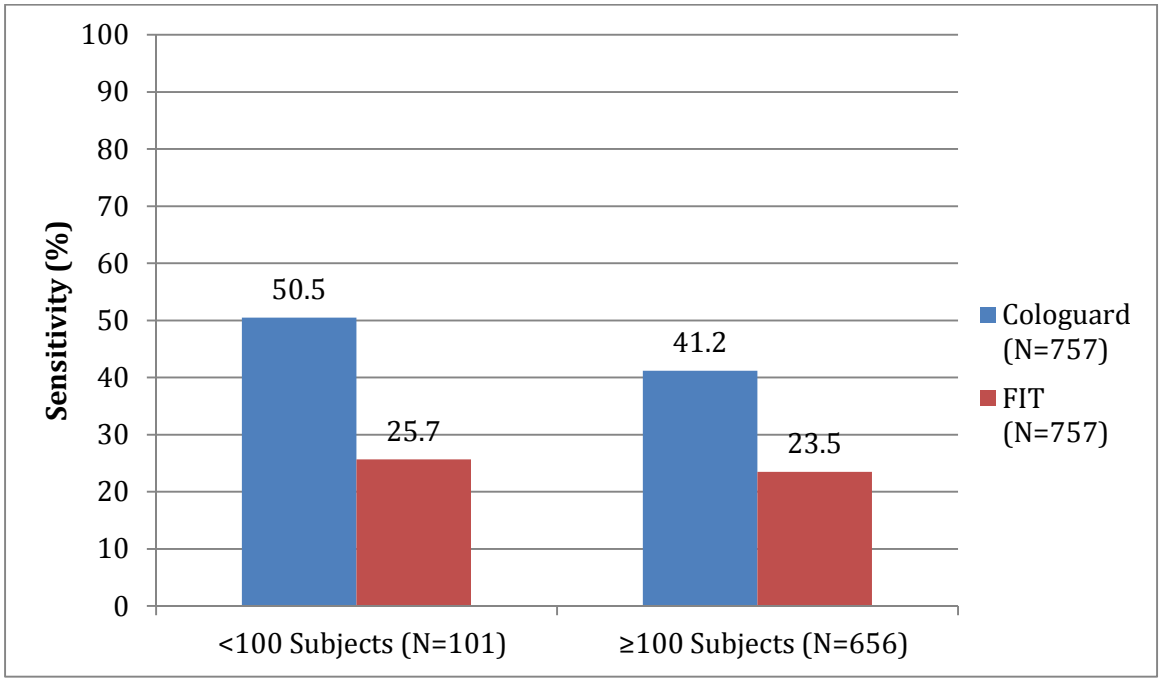
Cologuard sensitivity for CRC also did not differ significantly by size of study site. Sensitivity for AA was slightly lower at sites with ⩾ 100 subjects, compared with sites with < 100 subjects. Results for FIT were similar, though CRC sensitivity was lower at large sites enrolling more than 100 subjects than at smaller sites.

Figure 32: *Cologuard* and FIT CRC (Category 1) Sensitivity by Site Size Secondary Effectiveness Subjects*



* Sensitivity calculated as number of CRC positives divided by subjects with CRC.

Figure 33: *Cologuard* and FIT AA (Category 2) Sensitivity by Site SIze Secondary Effectiveness Subjects*

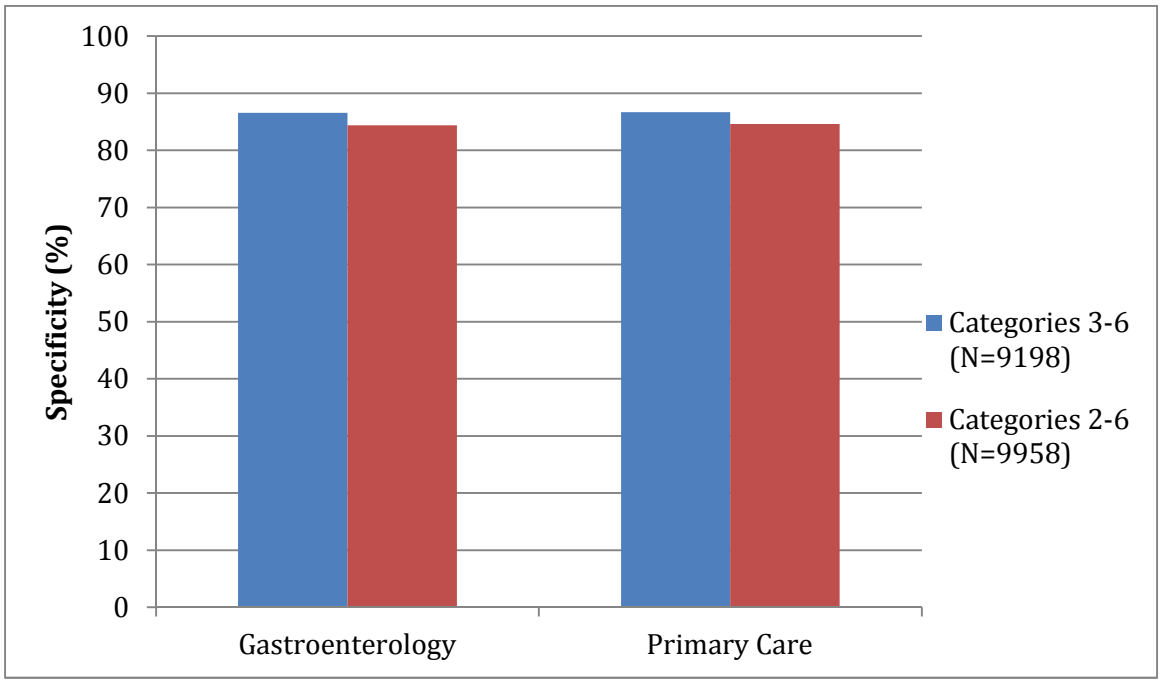


***** Sensitivity calculated as number of AA positives divided by subjects with AA.

Furthermore, with respect to sample processing laboratory, there were no notable differences in results, although sensitivity for AA was slightly varied.

As shown in **Figure 34**, *Cologuard* specificity was similar at primary care sites (86.7%), compared with colonoscopy sites (86.6%), when AA cases were excluded from the analysis (Categories 3-6). In the alternate analysis, where AA cases were included in the analysis (Categories 2-6), specificity again was similar at primary care sites (84.6%) compared with colonoscopy sites (84.4%).

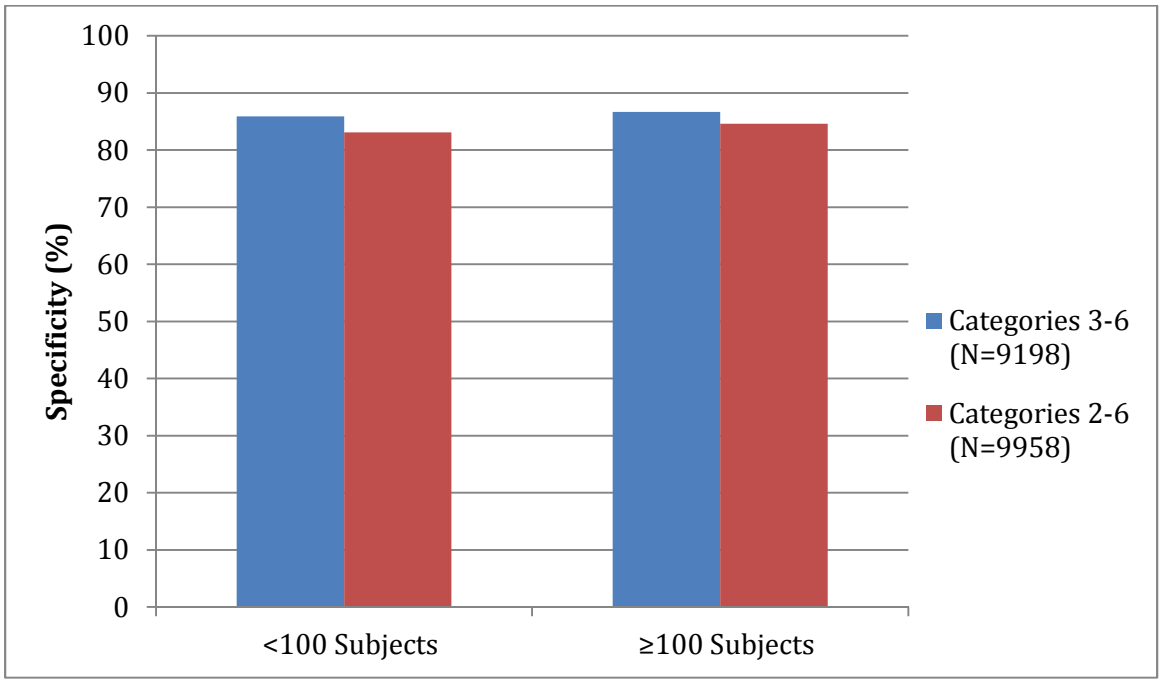
Figure 34: *Cologuard* Specificity by Point of Referral Site (Specificity Subsets: Categories 3-6 and 2-6) – Primary Effectiveness Subjects*



*Specificity calculated as number of negatives among subjects without CRC or AA.

With respect to size of study site, specificity was similar at both large and small sites, as shown in **Figure 35**. When AA cases were excluded from the analysis (Categories 3-6), rates at each individual lab were similar to the overall specificity for *Cologuard* (86.6%) in the study. In the alternate analysis, in which AA cases were included from the analysis, specificity ranged from 81.9% to 86%.

Figure 35: *Cologuard* Specificity by Site Size (Specificity Subsets: Categories 3-6 and 2-6) – Primary Effectiveness Subjects*



*Specificity calculated as number of negatives among subjects without CRC or AA.

7.8.3 Safety Analyses

As noted previously, AEs were not expected in the DeeP-C study. There were no reported SAEs, and only 4 AEs were reported. One subject broke a fingernail when trying to open the collection kit, one subject cut his/her right thumb by using a knife (not supplied with the kit) to open the preservative bottle that accompanied the collection kit, one subject fell during stool collection and suffered leg pain as a result, and one subject experienced a sprained hand during sample collection. All of the AEs experienced in the study were categorized as "mild" events, per the protocol definitions of AE severity. None of the events led to the subject discontinuing the study.

Additionally, one subject died prior to undergoing colonoscopy. The subject met all eligibility criteria and successfully collected a stool sample. The subject did not present for the subsequent colonoscopy and it was discovered by the study site that the subject had died, due to narcotic and ethanol intoxication. This event was deemed unrelated to the study and not captured as an AE because it occurred outside of the AE reporting period (AEs were only captured during the sample collection period and the subject had already returned the stool sample).

8.0 POST APPROVAL STUDY PLAN

After discussion with FDA, Exact Sciences had developed a post-approval study plan that is provided with this summary. The objective of the study is to collect longitudinal data on subjects prescribed *Cologuard* and to assess the risk of CRC/AA among those with a positive Cologuard test at the third year of follow-up (T3) compared to baseline (T0).

The study will enroll approximately 1,830 subjects at approximately 20 sites to achieve a minimum of 946 subjects at the Year 3 visit. Key eligibility criteria include subjects between the ages of 50 and 84 (inclusive) who have not had a colonoscopy in the last 9 years and are at average risk for development of colorectal cancer. The eligibility criteria are generally consistent with the pivotal study.

Once enrolled, subjects will undergo a *Cologuard* test (T0). All positive *Cologuard* results will be referred to colonoscopy. Due to the potential that tissue will be removed during the colonoscopy procedure, even among "negative" subjects, all subjects undergoing a colonoscopy will be discontinued from the study. All remaining subjects will be assessedat the first and second year of follow-up to evaluate whether any changes in medical history warrant additional screening by colonoscopy. At the third year of follow-up, all remaining subjects will undergo a *Cologuard* test (T3) and a colonoscopy.

The primary endpoint for this study is to assess the risk of CRC/AA among those with a positive *Cologuard* test at the third year of follow-up (T3) compared to baseline (T0).

The secondary endpoints are to evaluate the distribution of colorectal epithelial lesions (by Category) among positive Cologuard subjects at T0 and at T3, and to evaluate the predictive values of a positive Cologuard at T0 and at T3.

The study will provide longitudinal data that will begin to evaluate performance of the test over time.

9.0 RISK-BENEFIT ANALYSIS

Colorectal cancer (CRC) is the second leading cause of death from cancers affecting both men and women in the United States. Screening has been shown to reduce incidence and mortality from CRC.[76] According to a recent CDC publication, 90% of people live 5 or more years when cancer is identified early due to screening.[77]

Current guidelines for CRC screening in the average-risk population recommend regular screening of both men and women starting at age 50. Unfortunately, recent estimates suggest that approximately 1 in 3 adults are not undergoing screening as recommended.

Stool DNA (sDNA) testing is a non-invasive method designed to detect tumor-specific DNA mutations and epigenetic alterations and requires only a single stool sample. sDNA testing detects molecular markers of altered DNA that are contained in the cells shed by cancerous tumors and large lesions into the large bowel lumen. Unlike the blood markers that are intermittently found in stool, the DNA markers are released from cells that regularly and continuously slough from the lining of the colon into the stool. Through the use of selective enrichment and amplification techniques, sDNA tests are designed to detect even very small amounts of the DNA markers to identify CRC or AAs in the colorectal region.

Cologuard is an sDNA test designed to provide an additional screening option for CRC by detecting DNA and hemoglobin markers associated with colorectal neoplasia. Risks associated with *Cologuard* are similar to any non-invasive CRC screening test. There are no known direct risks to patient health associated with use of the *Cologuard*, however, a false positive or false negative result could potentially impact patient management. Specifically, a false positive result could result in an additional invasive screening procedure, such as colonoscopy, and thus expose patients to the attendant risks associated with such a procedure. A positive result on *Cologuard* should lead to a diagnostic colonoscopy procedure, which is itself a standard of care CRC screening test. Those subjects without indicia of disease upon subsequent colonoscopy (e.g. a *Cologuard* false positive) would return to the screening pool and undergo subsequent surveillance at regular intervals as appropriate to their risk level, per current practice guidelines.

Similarly, a false negative result with *Cologuard* could potentially impact patient management by potentially delaying diagnostic colonoscopy and potentially delaying diagnosis of disease. The risk of delayed diagnosis due to a false negative with *Cologuard* for CRC is relatively low. This study allows an estimate of 1 CRC false negative in every 2,000 patients screened, which is reasonable in light of estimates for colonoscopy screening[78] and much lower than of FIT, at 3.4/2,000 patients screened.

Cologuard offers significant benefit with respect to CRC screening. *Cologuard* was highly sensitive for CRC and had a statistically significant advantage over FIT in this regard. Sensitivity of *Cologuard*

---

[76] Lansdorp-Vogelaar I, van Ballegooijen M, Zauber AG, Habbema J, and Kuipers EJ. (2009) *J Natl Cancer Inst*; 101:1412-1422; *Winawer SJ, Zauber AG. (2002) The Advanced Adenoma as the Primary Target of Screening. Gastrointest Endosc Clin N Am. 12(1):1-9.*

[77] Colorectal Cancer Tests Save Live: The best test is the test that gets done. *CDC VitalSigns* November 2013, available at http://www.cdc.gov/vitalsigns/colorectalcancerscreening/?mobile=noconte (accessed November 15, 2013)

[78] Studies suggest that colonoscopy misses 17% of lesions ≥ 1 cm in size. Lieberman DA, Rex DK, Winawer SJ, Giardiello FM, Johnson DA, Levin TR. United States Multi-Society Task Force on Colorectal Cancer (2012). Guidelines for colonoscopy surveillance after screening and polypectomy: a consensus update by the US Multi-Society Task Force on Colorectal Cancer. *Gastroenterology* 143(3):844-57.

for CRC was 92.3% (60/65), compared with 73.8% (48/65) for FIT, a nearly 20 percentage point absolute improvement over FIT. Importantly, *Cologuard* outperformed FIT with respect to detection of early-stage cancers, when the potential for effective treatment is the greatest.

Cologuard was also superior to FIT for the detection of AAs (42.4% for *Cologuard* compared with 23.8% for FIT). Importantly, *Cologuard* detected a higher proportion of AAs with carcinoma *in situ*/high grade dysplasia (69.2%, compared with 46.2% for FIT), the type of lesion most likely to progress to CRC. *Cologuard* also demonstrated a significant advantage in sensitivity for serrated lesions, a lesion that has been increasingly linked to CRC development. Specificity of *Cologuard* was also high, meeting the pre-specified threshold for primary endpoint success.

Additionally, *Cologuard* sensitivity was universally high across various demographic groups. *Cologuard* sensitivity for CRC was consistently high across all age groups, ranging from 88.9% to 100.0% for subjects 65 years of age and older. Sensitivity of *Cologuard* for CRC was high, regardless of gender (100.0% for males, 83.9% for females), and was generally high across racial/ethnic groups, despite the small number of CRC cases in some groups.

Meanwhile, the risks associated with *Cologuard* are not significant, in that even patients with a false negative result should be directed to return periodically per current screening guidelines and undergo another CRC screening event in the future, as recommended for this population. Adverse events associated with *Cologuard* were minimal in the DeeP-C study and related largely to minor events reported by 4 of 12,776 (0.03%) patients during stool sample collection.

In summary, the study demonstrated that *Cologuard* was highly sensitive and specific for CRC. The analysis also demonstrated that *Cologuard* was superior to a currently available FIT product for both CRC and AA detection. Considering the risks compared to the benefits of screening with *Cologuard*, Exact Sciences believes that *Cologuard* provides an additional, important screening option for CRC and AA. All information to date demonstrates a favorable risk-benefit profile for *Cologuard*.

10.0 OVERALL CONCLUSIONS

The information summarized above and included in the company's PMA submission to FDA demonstrates the safety and effectiveness of *Cologuard*, based on the results of the DeeP-C study. Data are presented for 10,023 subjects with complete data for the analyses. *Cologuard* demonstrates a clinically meaningful sensitivity (92.3%) for CRC and specificity (86.6%), and the DeeP-C study powerfully demonstrating that *Cologuard* is statistically significantly superior to FIT testing for detection of both CRC and AA.

In addition to meeting the primary and secondary endpoints of the study. *Cologuard* consistently demonstrated strong performance across cancer stages and lesion sizes. In particular, *Cologuard* sensitivity was particularly strong for Stage I thorugh III CRC, stages that are most likely to respond well to treatment and have the highest survival rates. In addition, Cologuard sensitivity for AA represented a clear advantage over FIT, particularly for larger adenomas. Additionally, Cologuard sensitivity for adenoma with carcinoma in situ/high grade dysplasia, lesions at high risk of developing into CRC, was 69.2% compared with 46.2% for FIT.[79] *Cologuard* also provides an advantage in sensitivity for detection of serrated lesions, a lesion that has been increasingly linked to CRC development. Cologuard sensitivity for serrated lesions was 43.0%, compared with 5.1% for FIT.

As a well-controlled investigation, the DeeP-C pivotal clinical study, constitutes valid scientific evidence, as defined in 21 C.F.R. §860.7(c)(2), upon which the agency can make a determination of the safety and effectiveness of the device. The pivotal study results demonstrate a reasonable assurance that *Cologuard* is safe (as defined in 21 C.F.R. §860.7(d)(1)). The probable benefits to health from use of the device for its intended uses and conditions of use, when accompanied by adequate directions and warnings against unsafe use, outweigh any probable risks. In addition, the study results demonstrate the absence of unreasonable risk of illness or injury associated with the use of the test for its intended uses and conditions of use. Exact Sciences also believes that the pivotal study provides a reasonable assurance that *Cologuard* is effective (as defined in 21 C.F.R. §860.7(e)(1)) because, in a significant portion of the target population, the use of *Cologuard* for its intended uses and conditions of use, when accompanied by adequate directions for use and warnings, provides clinically significant results.

For these reasons, the company believes that the clinical data support approval of *Cologuard* as an adjunctive screening test for the detection of colorectal neoplasia associated DNA markers and for the presence of occult hemoglobin in human stool. In granting expedited review status for the *Cologuard* PMA, FDA has recognized that *Cologuard* is intended for use in screening for a life-threatening disease, CRC, and that *Cologuard* represents a breakthrough technology that offers significant, clinically meaningful advantages over existing non-invasive screening options. *Cologuard* will provide an important additional screening option for patients at normal risk of developing CRC.

[79] Toll AD, Fabius D, Hyslop T, Pequignot E, DiMarino AJ, Infantolino A, Palazzo JP. (2011) Prognostic significance of high-grade dysplasia in colorectal adenomas. *Colorectal Dis*;13(4):370-3.